CROHN'S DISEASE:

Diagnostic and prognostic indicators with special reference to granulomas.

Dissertation for the M.Med (Anat. Path.) degree

Submitted by DR. M.D. FORDER.

ACKNOWLEDGEMENTS:

Dr. S.K. Price	Department of Anatomical Pathology, GSH and UCT Medical School - my supervisor, for his help and encouragement.
Prof. J.P. Wright	GIT Clinic, Groote Schuur Hospital - for providing both access to his database and the clinical information including the NCCD Crohn's Disease Activity Index score.
Dr. S. Isaacs	Dept. of Medical Informatics, GSH - for performing the statistical analyses on my database.

CONTENTS

CHAPTER 1

INTRODUCTION

> "I can only regret that the aetiology of the condition remains in obscurity but I trust that ere long further consideration will clear up the difficulty"
>
> Dalziel, T.K. (1913)
> British Medical Journal, ii, 1668

It is well known that Crohn's disease is named after the first of three co-authors whose names were arranged alphabetically at the head of their clinical and pathological study of regional ileitis which appeared in 1932 (1). It is equally well known to gastroenterologists and medical historians that this important paper was not the first description of this condition.

Historians have traced descriptions of diseases presenting with rectal bleeding and weight loss back to the ancient

Greeks. Although these may have been cases of dysentery, Crohn's disease could explain these findings (2).

In 1769, Morgagni performed a post mortem on a 34 year old man who had died of intestinal perforation and peritonitis. He found the terminal ileum and colon to be inflamed, ulcerated and strictured, with large mesenteric lymph nodes. Although this illness was attributed to intestinal tuberculosis, Crohn's disease remains a strong diagnostic candidate (3).

In 1813, a report of thickening and stricture of the terminal portion of the ileum was given before the Royal College of Physicians at London by Combe and Saunders : the ileum was "contracted for the space of three feet to the size of a turkey quill" (3).

Sir Kennedy Dalziel, in 1913, described 9 patients with chronic interstitial enteritis. Dalziel's description is still an accurate exposition of the features of the syndrome: "The affected bowel gives the consistence and smoothness of an eel in a state of rigor mortis, and the glands though enlarged are evidently not caseous".

When considering prognosis and treatment, Dalziel states "As far as I am aware, the prognosis is bad except in cases where the disease is localised, and even then seems rather hopeless unless operation be had recourse to ".(4)

The modern history of granulomatous disease of the intestinal tract begins with the classic paper by Crohn, Ginzburg and Oppenheimer on their 14 cases of regional ileitis (1). In their paper they stressed that there are no diagnostic microscopic features of this disease. The presence of giant cells and granulomas, in their opinion, was an accidental finding due to the inclusion of small particles of vegetable matter that had become entrapped in the ulcers, entered lymphatics and elicited a foreign body reaction.

Since this original description it took some thirty years before criteria of colonic involvement by Crohn's disease were described (5). Thus, it was recognised that this syndrome could affect the whole gastrointestinal tract and increasing numbers of reports of Crohn's disease appeared in the literature.

Initially, the diagnosis of Crohn's disease was made on the basis of clinical features and an absence of tuberculosis and other infectious conditions. However, as more cases were recognised, so pathological criteria were added to the definition (6). As alluded to earlier, the pathological findings are often non-specific and so endoscopic and radiological techniques have added new criteria for the diagnosis (7).

The aetiology and pathogenesis of Crohn's disease continues to baffle researchers to this day. Early workers were convinced that the aetiological agent was carried within the faecal stream (8). A later hypothesis suggested that Crohn's disease arose from the inability of the macrophage to degrade a variety of normal gut luminal constituents (9).
However, it appears that the pathogenesis revolves around a complex interplay between genetic and environmental factors, although both are poorly understood. A genetic predisposition for Crohn's disease was suggested in studies from Sweden (10). and Biemond et al (11) showed a relative risk of 1.25 for Crohn's disease in people with HLA-A2 antigens.

There is still no unified concept of aetiology and pathogenesis, although recent microbiological studies have stimulated interest in an infective aetiology (12). Most authors consider dietary factors to be important and an excess of smokers has been reported among patients with Crohn's disease (13).

Immunological mechanisms and auto-immunity have been considered in the pathogenesis and numerous autoantibodies have been detected in the serum of Crohn's disease patients. These, however, are thought to represent epiphenomena.
The demonstration of human mucosal antigens specifically recognised by antibodies in the serum of patients with Crohn's disease and activity of both serum and circulating lymphocytes

against epithelial antigens in mouse intestine is more direct evidence for an element of autosensitisation (14). The possibility that Crohn's disease represents a diffuse disorder affecting the whole of the gastrointestinal tract has been suggested by both physiological and immunological studies of apparently uninvolved intestine with the disease (15,48). Mucosal abnormalities leading to increased permeation of antigens across the mucosa have been suggested as mechanisms that might potentiate a state of chronic inflammation (15). However, these findings have been demonstrated in patients with other diseases such as coeliac disease and are therefore non-specific (18).

The pathogenesis of the extra intestinal manifestations of Crohn's disease is equally obscure. Immune complex deposition has been postulated as one possible mechanism but the presence of immune complexes has not been demonstrated (16). Increased circulating concentrations of cytokines, especially interleukin I, have been demonstrated in active inflammatory bowel disease and are known to be responsible for damage to tissues. But, once again these findings are non-specific (16).

Recently a more controversial mechanism has been postulated to explain the features of Crohn's disease. The authors suggest that the pathogenesis is related to multifocal gastrointestinal infarction and demonstrated occlusive fibrinoid lesions of arteries supplying areas of intestine

affected by Crohn's disease. It is suggested that the clinical and histological features can be explained by this pathogenetic mechanism.(17).

Since its acceptance as a separate entity, the pathology of Crohn's disease has been well described in numerous articles in the literature (5,6,21,61,65). Macroscopically the affected bowel has been likened to a hosepipe due to its rigid unyielding consistency as a result of marked mural thickening. The luminal surface may show a variety of patterns, the classical being the so called "cobblestone" appearance. Other areas show a more serpiginous type of ulceration often combined with areas of stricture. Fissuring ulcers are also commonly seen and probably represent the forerunners of the fistulae which may develop.
Another characteristic macroscopic finding is the presence of "skip-lesions" with diseased bowel alternating with areas of more normal appearing bowel. This discontinuous inflammatory process is an important endoscopic clue in the differential diagnosis of Crohn's disease.

Microscopically, the main features include the transmural nature of the inflammatory infiltrate with prominent lymphoid hyperplasia forming aggregates especially in the deeper layers. Oedema and fibrosis of the submucosa are also often present with neural hypertrophy a well described feature. The mucosa may show considerable variation in the degree and

extent of features including inflammatory cell infiltration, villous abnormalities, pyloric metaplasia of the crypts of Lieberkuhn and ulceration. The presence of epithelioid granulomas is still considered to be the microscopic hallmark of this disease. However, in the absence of these, the diagnosis is fraught with difficulties.

Early changes in Crohn's disease are important to recognise and features described in the small intestine include changes in villous morphology (18), goblet cell hyperplasia (8,18) and focal necrosis of mucosal epithelium.(19). Aphthous ulceration, related to mucosal or submucosal lymphoid aggregates, is regarded as one of the more readily identifiable early changes in Crohn's disease.(18,20,21) However, this feature cannot by itself be regarded as diagnostic as it is seen in other inflammatory bowel diseases, such as Campylobacter colitis and yersiniosis.(18,22,23)

Examination of material by electron microscopy has shown increased mucous secretion (24) and axonal degeneration. (25)

The advent of the endoscopic biopsy has allowed the histopathologist to study the disease process without reliance on resection specimens. By performing colonoscopy the physician is able to visualise directly the mucosal surface and so obtain an image of the spatial variation of the disease process (i.e. right vs. left sided colitis). Geboes and

Vantrappen (26) have shown that colonoscopy is superior to radiography in defining certain mucosal abnormalities and for recognising the segmental distribution of disease. The relative ease of procuring these small biopsies allows the endoscopist to sample widely from macroscopically normal and abnormal mucosa, and to repeat the biopsies in different phases of disease activity. This is particularly valuable in inflammatory bowel disease as it often takes more than one biopsy to distinguish between the different inflammatory conditions that can so easily mimic each other clinically. The limitations of the mucosal biopsy however, must be appreciated and it should be realised that certain helpful features, such as depth of mural inflammation or presence of fissures cannot be detected (27). Features which favour Crohn's disease include granulomas, lymphoid hyperplasia - particularly when it extends into the submucosa and causes a disproportionate increase between the submucosal and mucosal inflammatory infiltrate (28,29,30) - and a patchy infiltrate due to variations in extent and degree of chronic inflammatory cells in the lamina propria, both within a single biopsy and in separate biopsies. Based on counts of inflammatory cells, it has been suggested that macrophages in the lamina propria are more prominent in Crohn's disease than other inflammatory conditions (31). Most other findings, such as crypt abscesses, pseudopolyps, submucosal oedema, tissue eosinophils, Paneth cell metaplasia and endocrine cell hyperplasia are non-discriminatory (32,33). Isolated giant cells without definite

granulomas are occasionaly present and may simply reflect reactions to luminal contents after loss of mucosal integrity (1,39).

The finding of granulomas in both biopsy and resection specimens, in the absence of other apparent causes is very suggestive of Crohn's disease. The incidence of granulomas in Crohn's disease however, varies considerably depending on the stage of the disease (34), the anatomical location within the gastrointestinal tract, the diligence with which the histopathologist searches for granulomas (35,36) and whether the patient was receiving medical treatment (prednisone and/or sulphasalazine) (37). Realising this, different authors have reported varying figures as to the incidence of granulomas in resection specimens and in mucosal biopsies. Price and Morson state that non-caseating sarcoid - like granulomas are found in up to 60 per cent of cases of surgical resections (38). Goldman and Antonioli emphasize that granulomas are detected in only about 50 per cent of cases of Crohn's disease when surgically resected bowel wall is available for examination (39).

The incidence in colonic and rectal mucosal biopsy specimens is more limited. In patients with disease limited to the small bowel, colonoscopic biospy of the uninvolved colonic mucosa reveals granulomas in 5 - 10 per cent of cases, whilst with

gross colonic involvement, granulomas are present in up to 25 per cent of cases (39).

Schmitz - Moormann et al. found that studying only one biopsy, a granuloma was found in 11 per cent of endoscopic biopsies, while the study of 6 biopsies increased the incidence of granulomas to 47 per cent (40). In the same way, the percentage positivity grew with the number of endoscopies - from 23 per cent for one endoscopy to 48 per cent for four endoscopies. From their results, they postulated that granulomas always occur in Crohn's disease, but only temporarily and not in all intestinal regions.

The value of rectal biopsies in the diagnosis of Crohn's disease has also been widely documented (27 - 30,41 - 47,64,66,67). The probability of finding granulomas in rectal biopsies varies from 20 per cent (28, 51) to 39 per cent (42). In one series, Lockhart-Mummery and Morson found granulomas in 16 out of 19 patients who had rectal biopsies (61). The considerable variation in incidence presumably relates to differences in the number of biopsies examined from each patient and in the number of histologic sections examined. In order to extract the maximum benefit from a rectal biopsy, studies have been done to determine the usefulness of serial sectioning. Surawicz et al recommend a partial serial sectioning of at least two rectal biopsies with at least 90 sections (36). Petri et al emphasize that only total serial sectioning will ensure that granulomas are not overlooked and in their series they examined an average of 623 sections from

each biopsy (49). In spite of this they still only encountered granulomas in 28 per cent of their cases.

The variation of granulomas from region to region within the bowel has also been studied. In the small bowel there appears to be a low incidence, which increases in the colon and rectum, with the highest incidence being found in the anus (50). The explanation for this is not clear. Theories suggested include that it may be due to environmental differences - there may be increasing concentrations of granuloma-forming material distally. However, the regional variation in granuloma incidence would be more likely to correlate with the distribution of intestinal involvement which does not occur. Another explanation could be that different areas of the bowel exhibit inherent differences in their ability to respond to the causative agent.
Other authors have indicated that the number of granulomas decreases with long standing illness (40,50) and that ileal Crohn's disease presents clinically much later in the disease course than more distal disease.
None of these explanations appears to be entirely satisfactory.

The clinical course of Crohn's disease is punctuated by a cyclical waxing and waning of disease activity. There appears to be a relentless progression of the disease to the extent that the vast majority of patients will eventually require

surgical intervention (53,54). In a study from Cape Town it was shown that 46 per cent of Crohn's patients come to resection within 5 years and 23 per cent have a second resection within 5 years of the first resection (57). Because of the variability in the clinical course of Crohn's disease, numerous attempts have been made to define criteria which might predict the likelihood of disease progression in Crohn's patients. Initially, clinical indices were defined and correlated by the American National Co-operative Crohn's Disease (NCCD) Study Group (58). However, these did not appear to have any significant predictive value, possibly because the parameters included were mainly subjective ones. (eg. diarrhoea, abdominal pain and well-being). More objective criteria, such as laboratory data as well as radiological and colonoscopic features were then analysed as to possible predictive value.

Of more interest to the pathologist is the role of histological features in the prediction of Crohn's disease activity. Initially it was thought that histological features did not relate to clinical course (52). However, as the granuloma is considered to be of prime importance in the diagnosis of Crohn's disease (in the right clinical setting and after exclusion of other causes of granulomatous inflammation) it was inevitable that this feature became the focus of possible correlation with prognosis. Early studies did not demonstrate any differences in prognosis associated

with granulomas (8,42,52,54,55). However, in a series reported by Glass and Baker (56), the authors found that the presence of sarcoid-like granulomata inferred a better prognosis for the patient than those without granulomata. These findings were subsequently confirmed in a study from St. Marks Hospital and the presence of granulomas was considered to be analagous to tuberculoid leprosy, in which granuloma formation is associated with a higher degree of cell mediated immunity and a better clinical outlook (50).

The fact therefore remains that, at the present time, Crohn's disease is still an enigma. The aetiology and pathogenesis are obscure, the clinical findings and progression of the disease are unpredictable and the histological findings are often non-specific. With this in mind, this dissertation attempts to define and document the incidence of certain histological features at presentation in a population of Crohn's disease patients from Groote Schuur Hospital.

The main aim is to determine the incidence of granulomas in the study group as a whole, as well as to establish the distribution of granulomas within the bowel. A correlation between the presence of granulomas and the clinical activity of the disease (as assessed by the Crohn's Disease Activity Index) is also sought.

The incidence of other less specific microscopic changes is analyzed and the value of endoscopic and rectal biopsies in the diagnosis of Crohn's disease is discussed.

CHAPTER 2

MATERIALS AND METHODS

The records of 148 patients with known Crohn's disease were accessed from the data bank of the Crohn's Disease Clinic at Groote Schuur Hospital. The original presenting pathological specimen from each patient was then retrieved from the files of the Department of Anatomical Pathology, University of Cape Town. Second and subsequent specimens were not included in this study, eliminating therapeutic variables.

Each specimen had been formalin fixed, paraffin wax embedded and stained with haematoxylin and eosin according to standard histological procedures. The specimens were then divided into 3 categories :

(a) resection specimens (42 cases)

(b) colonoscopic biopsies - including rectal biopsies taken at the same time (45 cases)

(c) isolated rectal biopsies (61 cases)

Clinical data recorded for each patient included a Crohn's Disease Activity Index (CDAI) which coincided with the date that the pathological specimen was obtained. The CDAI was devised in conjunction with the American National Cooperative Crohn's Disease (NCCD) Study Group (58). This index quantitates a number of clinical symptoms and signs over a 7 day period. These variables include the general well-being of the patient (assessed by the attending physician), the extent

of abdominal pain, the number of soft/liquid stools, the presence of an abdominal mass, extra-intestinal manifestations and the amount of treatment required to control symptoms. Also included are more objective criteria such as the patient's body weight and the haematocrit. This clinical index has been used for a number of years at the Crohn's disease clinic at Groote Schuur Hospital and is an assessment of clinical "activity" at that time.

Index values of 150 and below are associated with quiescent disease; values above that indicate active disease and values above 450 are seen with extremely severe disease (58).

The histological parameters that were noted included:

[A] GRANULOMAS: (in all 148 cases)

Epithelioid granulomas : large sarcoidal-like accumulations of epithelioid macrophages without caseation necrosis (Figure 1). In some cases the granulomas had a more feathery, loose appearance, related to early paralymphatic formation, but these were still counted as epithelioid granulomas (Figure 2).

Microgranulomas : a small ill-defined collection of epithelioid macrophages usually measuring 125 to 200μm in diameter (Figure 3). They do not contain giant cells (43).

Isolated giant cells : a single giant cell without associated epithelioid cells.

[B] OTHER INFLAMMATORY CHANGES: (in 45 colonoscopic and 61 rectal biopsies)

Ulceration : loss of the surface mucosa with an underlying infiltrate of neutrophils.

Aphthous ulceration : an erosive lesion often related to a mucosal or submucosal lymphoid aggregate. There is an acute inflammatory exudate associated with the lymphoid aggregate (18) (Figure 4).

Lymphoid hyperplasia : an increase in collections of lymphoid cells resembling follicles, often at the base of a crypt.

Mucin depletion : an obvious decrease in the amount of mucin within goblet cells.

Cryptitis : an infiltrate of neutrophils within the crypt epithelium (Figure 5A).

Crypt abscess : extension of the neutrophils from the lamina propria through the crypt epithelium into the crypt lumen (Figure 5B).

Crypt distortion : disturbance of the normal straight tubular crypt architecture including crypt atrophy and crypt degeneration (Figure 5B).

Non-specific inflammation : more than a few neutrophils was considered indicative of "active" inflammation. Eosinophils are normally scanty in the large intestinal lamina propria and any more than the occasional one was considered pathological (23).

Plasma cells are normal constituents of the lamina propria and their presence was only considered to be pathological when two or more were found to be "touching".

The specimens containing non-specific inflammation were further categorised according to whether the infiltrate was superficial (affecting the upper half of the mucosa only), diffuse (a uniformly dense infiltrate) or patchy (a variation in density of the inflammatory cells between groups of crypts or between one crypt and the next. This is the histological counterpart of the gross "skip lesion").

Disproportionate submucosal inflammation : this refers to an excessive mixed infiltrate within the submucosa (Figure 6). This sign is only of value when the biopsy is from intact mucosa for, when ulceration is present the adjacent superficial submucosa may become involved. Disproportionate inflammation reflects the transmural nature of the inflammatory process.

The material recorded above was then analyzed according to the following general plan:-

[I] GRANULOMAS:

[i] Incidence of granulomas:

The overall incidence of granulomas as well as the incidence of epithelioid and microgranulomas in all 148 cases.

[ii] Distribution of granulomas in the layers of the bowel wall:

In the resection specimens, the presence of granulomas was noted according to their distribution within the bowel wall i.e. whether they were situated in the mucosa, the superficial or deep submucosa, muscularis propria or serosa.

[iii] Longitudinal distribution of granulomas in the large intestine:

The colonoscopic biopsies were divided into the longitudinal site from where they were taken i.e. caecum, ascending colon, transverse colon, descending colon, sigmoid and rectum. The presence of granulomas was entered accordingly.

[iv] Intramucosal distribution of granulomas:

For the colonoscopic and rectal biopsies, the granulomas were also recorded as to whether they occurred alone in the lamina propria or whether they were associated with a crypt, an ulcer or a lymphoid follicle.

[v] Correlation with Crohn's Disease Activity Index (CDAI):

All 148 cases could be divided into whether disease activity was present (CDAI > 150) or absent (CDAI < 150). For each separate group the presence or absence of granulomas had been recorded. Statistical significance was determined by creating a 2x2 table and comparing the percentage differences by using the chi-square test and from this deriving a p-value.
A p-value of less than 0.05 is statistically significant (68).

[II] COLONOSCOPY SPECIMENS:

[i] Granulomas:

The incidence of granulomas in colonoscopy specimens was compared with other series reported in the literature.

[ii] Inflammatory changes:

The incidence of other less specific inflammatory changes and the longitudinal distribution of these changes in the large intestine was noted i.e. a comparison of right vs left sided colonic involvement.

[iii] Diagnostic value of colonoscopic biopsies:

The number of cases in which inflammatory changes, other than granulomas, were specific enough to be confident in proposing a diagnosis of Crohn's disease.

[iv] Correlation of inflammatory changes with the CDAI:

A statistical analysis to determine whether signs of histological activity, as evidenced by ulceration, neutrophils in the lamina propria, cryptitis or crypt abscesses, has any correlation with clinical "activity", as assessed by the CDAI.

[III] RECTAL BIOPSIES:

[i] Granulomas:

The incidence of granulomas in rectal biopsies was established according to the anatomical location of the disease process. The cases were divided as to whether there was macroscopic involvement of the rectum or not. Those without rectal disease were separated into ileitis only, ileo-colitis or colitis only.

[ii] Inflammatory changes:

The incidence of less specific inflammatory changes in rectal biopsies was recorded. The findings were also tabulated according to the anatomical location of the disease process.

[iii] Diagnostic value of rectal biopsy:

An attempt was made to evaluate the role of the rectal biopsy in the diagnosis of Crohn's disease

[iv] Correlation of inflammatory changes with the CDAI:

As with colonoscopic biopsies, to compare the features of histologic activity with the assessment of clinical activity.

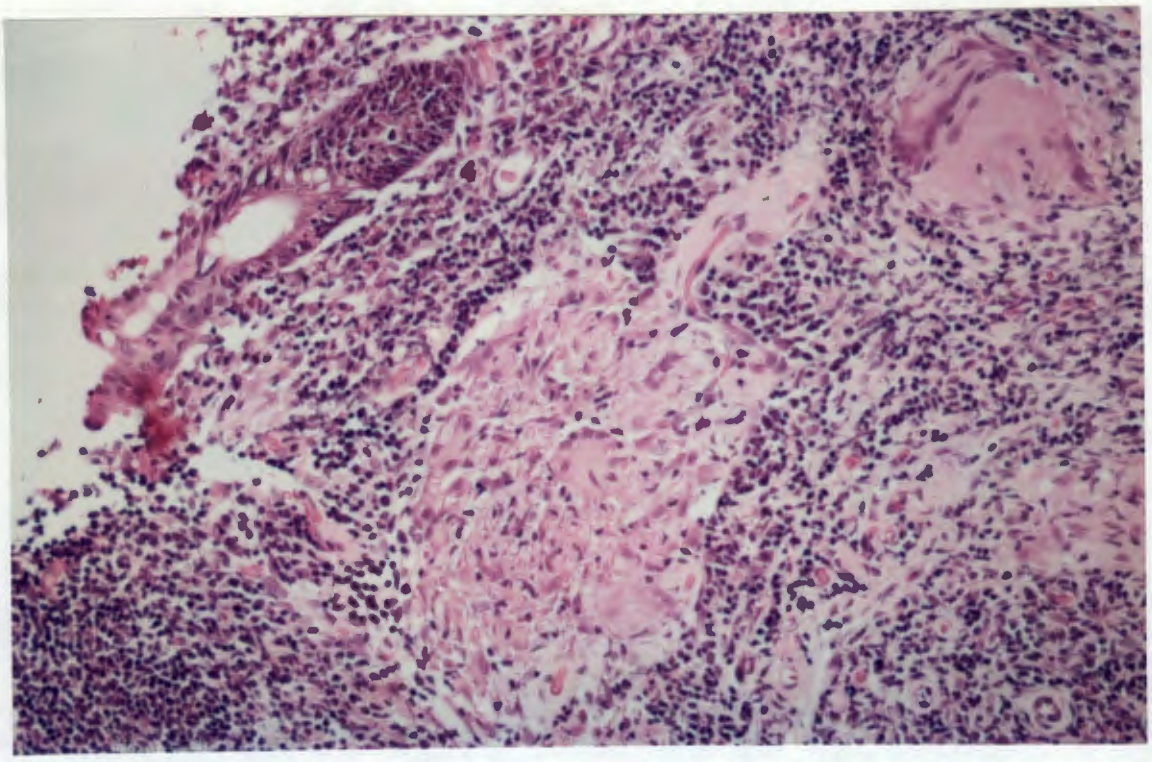


Figure 1: Epithelioid Granuloma, sarcoid-like.

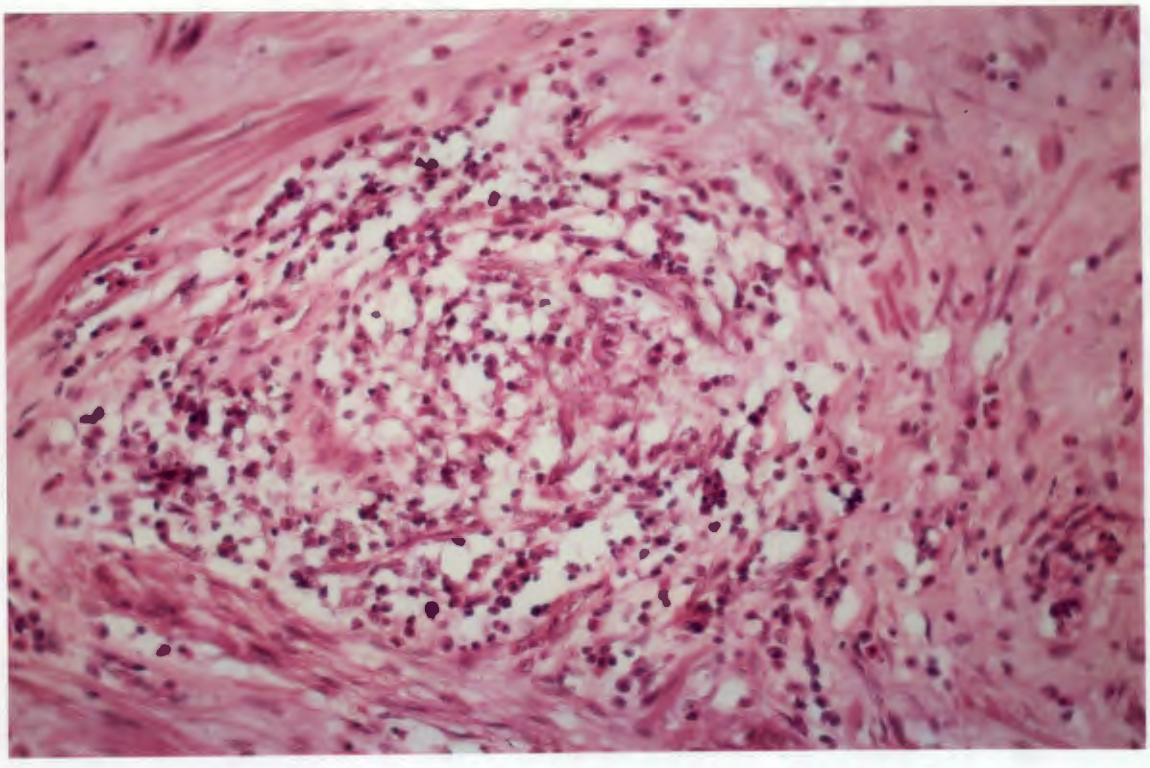


Figure 2: Epithelioid Granuloma - loose appearance.

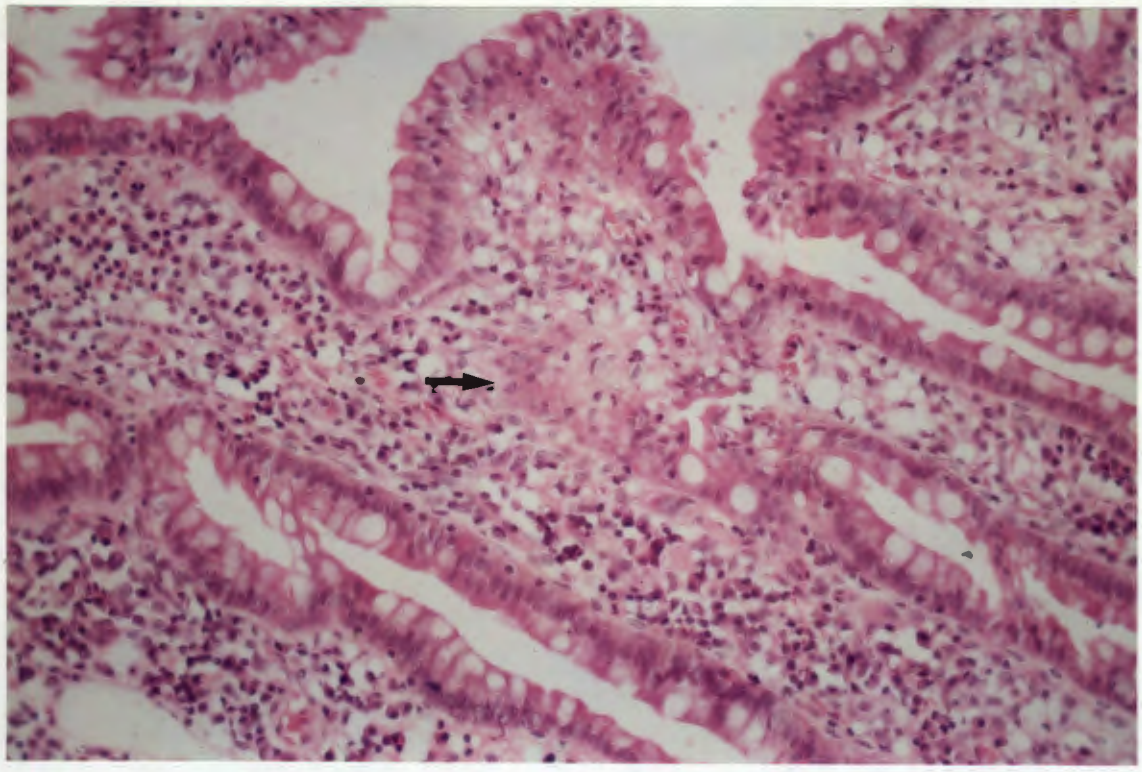


Figure 3: Microgranuloma - small ill-defined collection of epithelioid macrophages.

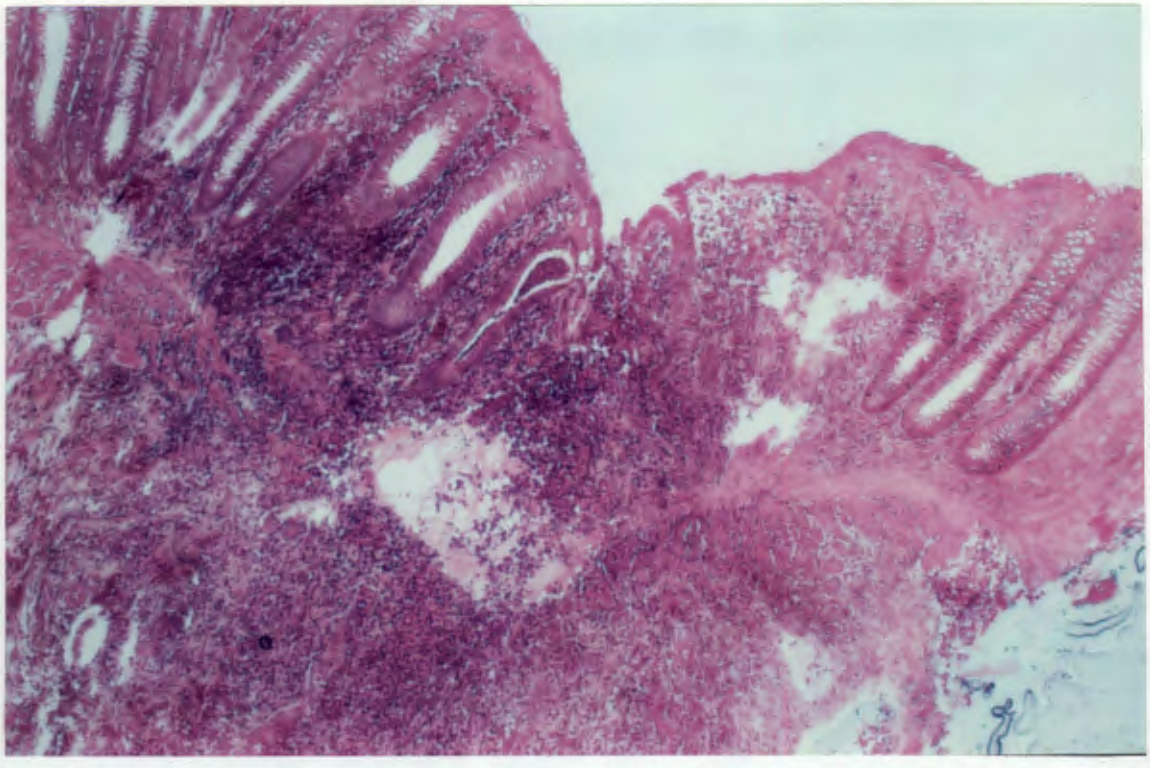


Figure 4: Early Aphthous Ulceration.

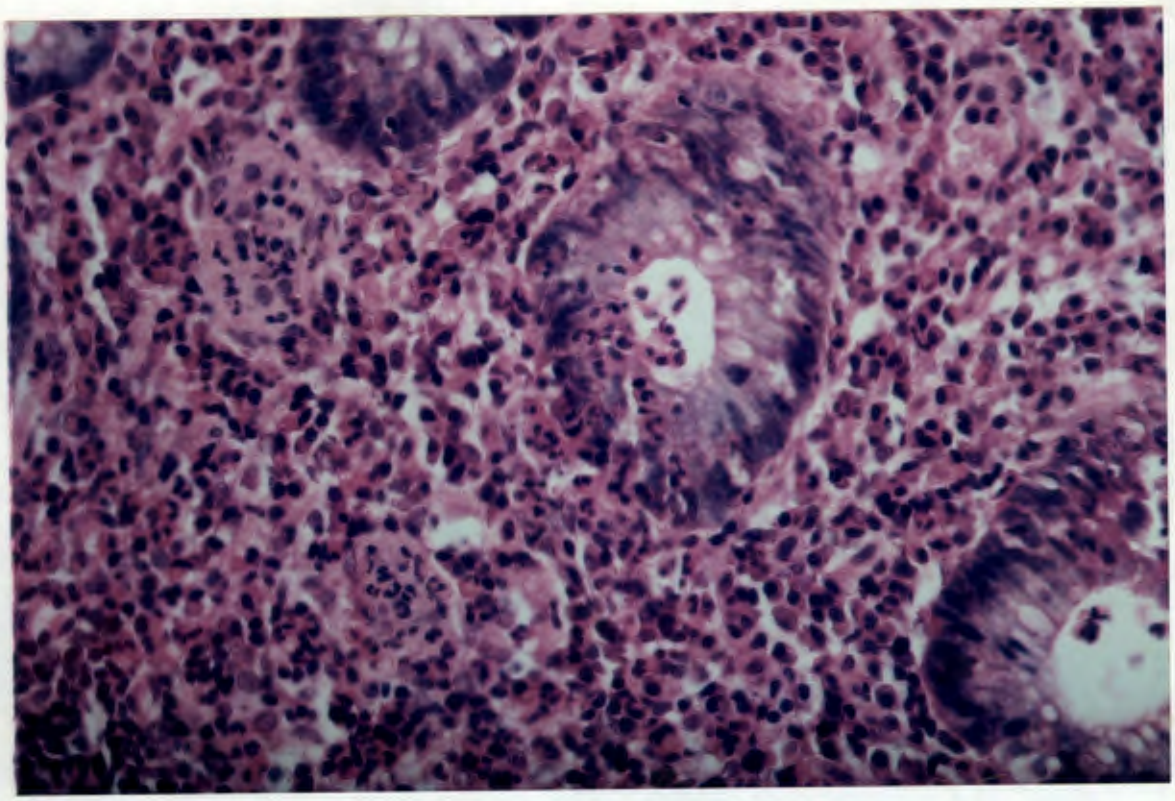


Figure 5: A. Cryptitis - Neutrophils in crypt epithelium.

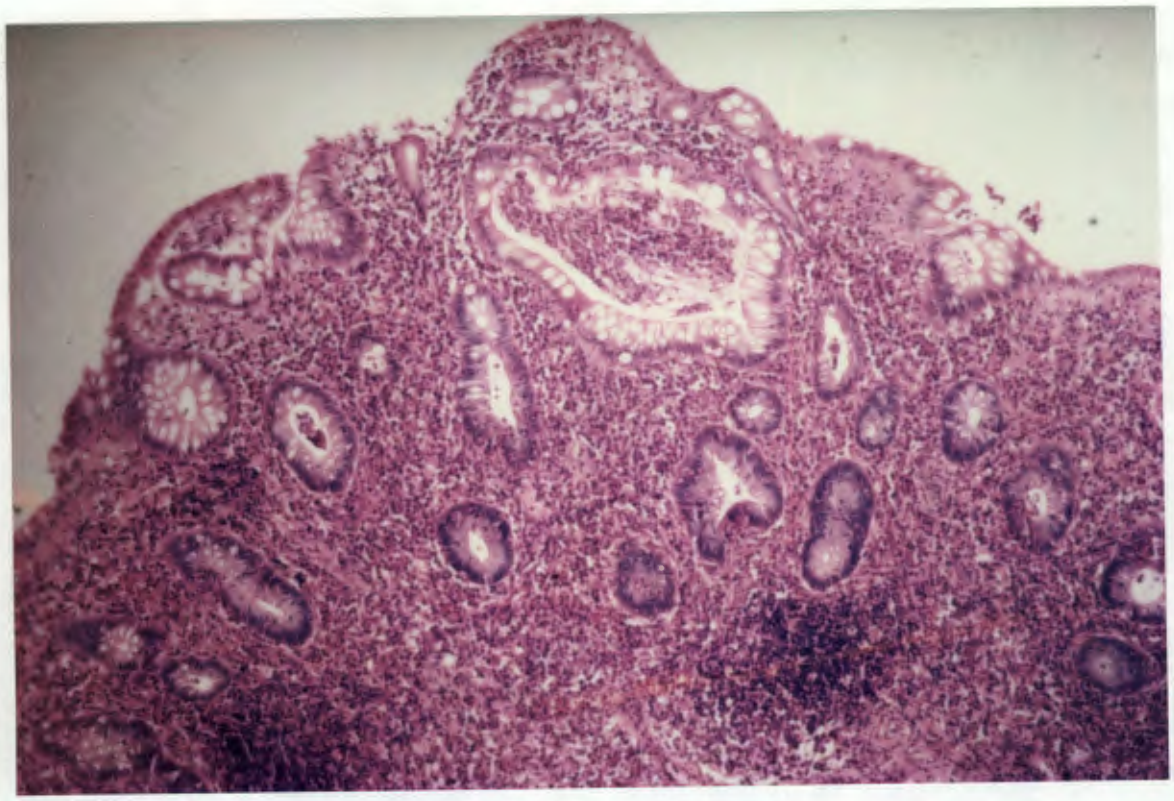


Figure 5: B. Crypt Abscess

Crypt Distortion.

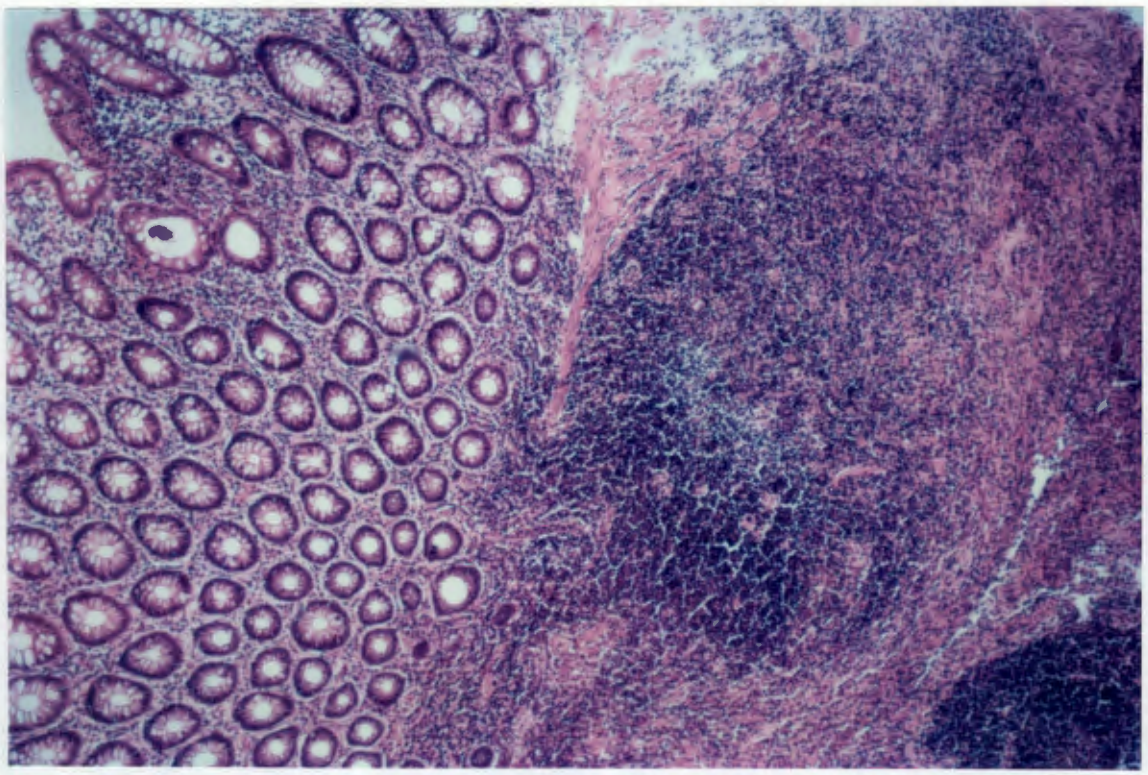
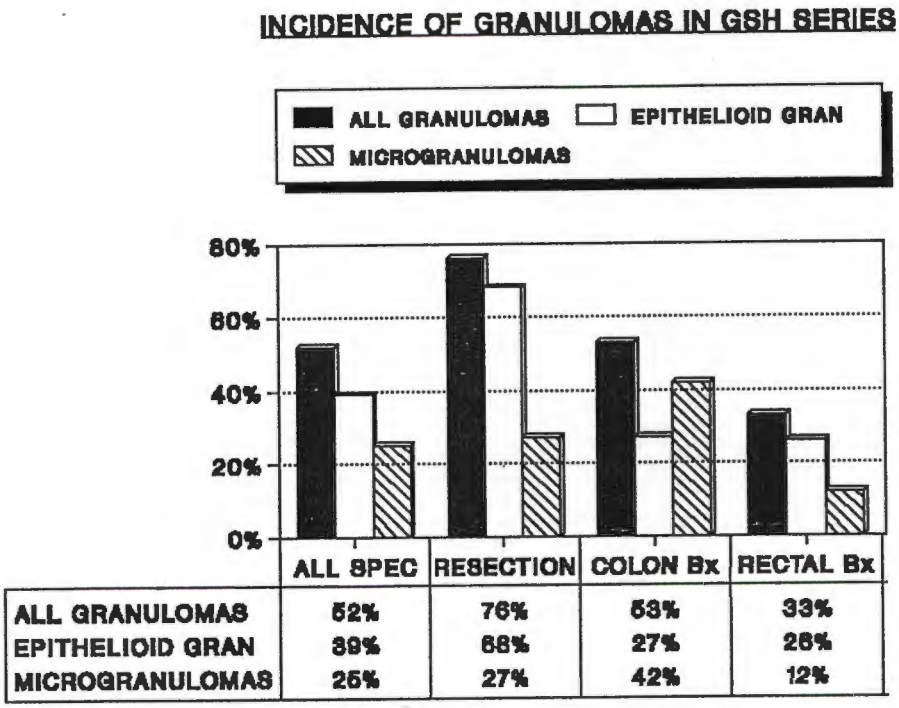


Figure 6: Disproportionate Submucosal Inflammation.

CHAPTER 3

RESULTS

[I] GRANULOMAS:



	ALL SPEC	RESECTION	COLON Bx	RECTAL Bx
ALL GRANULOMAS	52%	76%	53%	33%
EPITHELIOID GRAN	39%	68%	27%	26%
MICROGRANULOMAS	25%	27%	42%	12%

FIGURE 7:

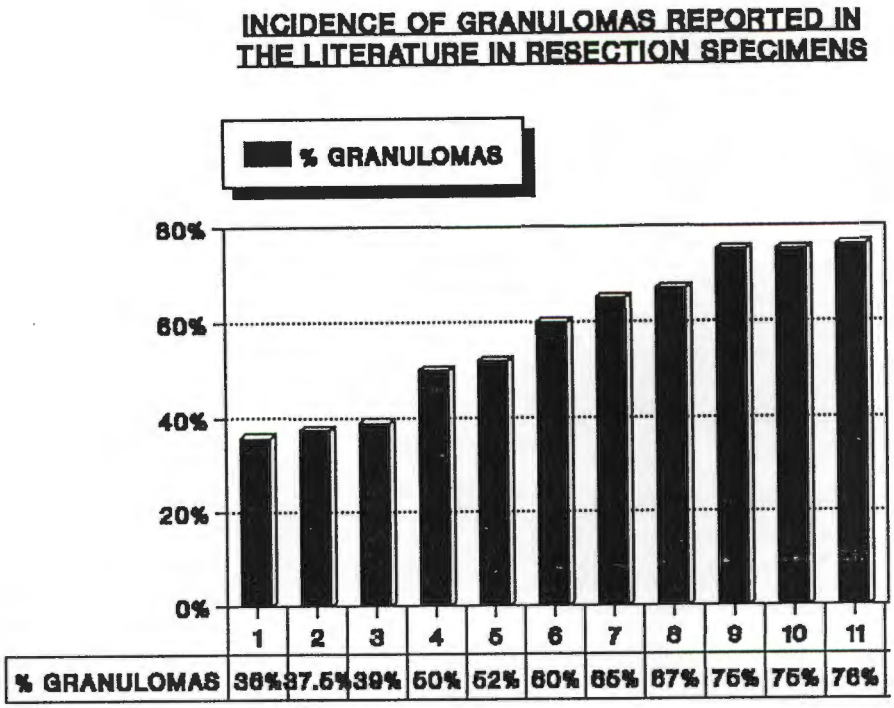
Figure 7: The overall incidence of granulomas in all specimens was 52 per cent. This compares favourably with figures quoted in standard pathology textboooks (18,38).

The graph in figure 7 shows a decreasing incidence of granulomas from resection specimens down to rectal biopsies.

As the majority of resection specimens were from the small intestine, this would seem to imply that there is a decreasing incidence of granulomas from the small intestine through the colon to the rectum. However, the high incidence in the small intestine is most likely due to the amount of material available in resection specimens for histological examination and not due to a true increase in incidence. This creates a false impression of the variation in longitudinal distribution of granulomas in the intestine. This topic is illustrated more thoroughly further on in the discussion (see figure 11).

The high incidence of microgranulomas in colonoscopic biopsies is a feature which has not been reported before.

FIGURE 8:

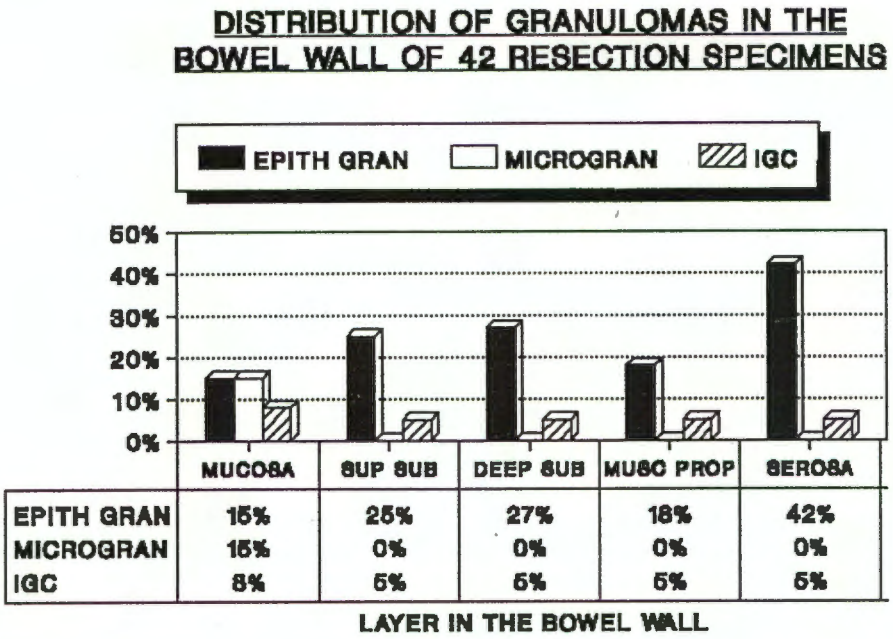


SERIES:	AUTHOR:	REFERENCE:
1:	Gump, et al	(54)
2:	Antonius, et al	(52)
3:	Van Patter, et al	(8)
4:	Jones-Williams	(6)
5:	Wolfson, et al	(55)
6:	Cook, Dixon	(60)
7:	Hadfield	(51)
8:	Glass, Baker	(56)
9:	McGovern, Goulston	(53)
10:	Lockhart-Mummery, Morson	(61)
11:	GSH	

Figure 8: The pathological specimens reported in most of these series consisted of both initial and recurrent specimens. In series 1,9 and 10 - Gump, et al (54), McGovern and Goulston

(53) and Lockhart-Mummery and Morson (61) - initial presenting specimens only were examined.

FIGURE 9:



(IGC = isolated giant cells; SUP SUB = superficial submucosa; DEEP SUB = deep submucosa; MUSC PROP = muscularis propria)

Figure 9: The incidence of granulomas appears to increase from the mucosal surface through the superficial and deep submucosa to the serosa. A noticeable dip, however, occurs in the muscularis propria. This could be due to a difference in

lymphatic drainage in the muscularis propria, as granulomas have been linked to lymphatic channels (8). The presence of lymphoid follicles in the external muscular layer varies from 15.7 per cent in Van Patter et al's series to 71 per cent in the series of McGovern and Goulston (8,53).

An original finding is the distribution of microgranulomas - which were only found in the mucosa and no other layers of the intestinal wall. This has not been reported in the literature before.

Isolated giant cells appear to be totally unrelated to granulomas.

FIGURE 10:

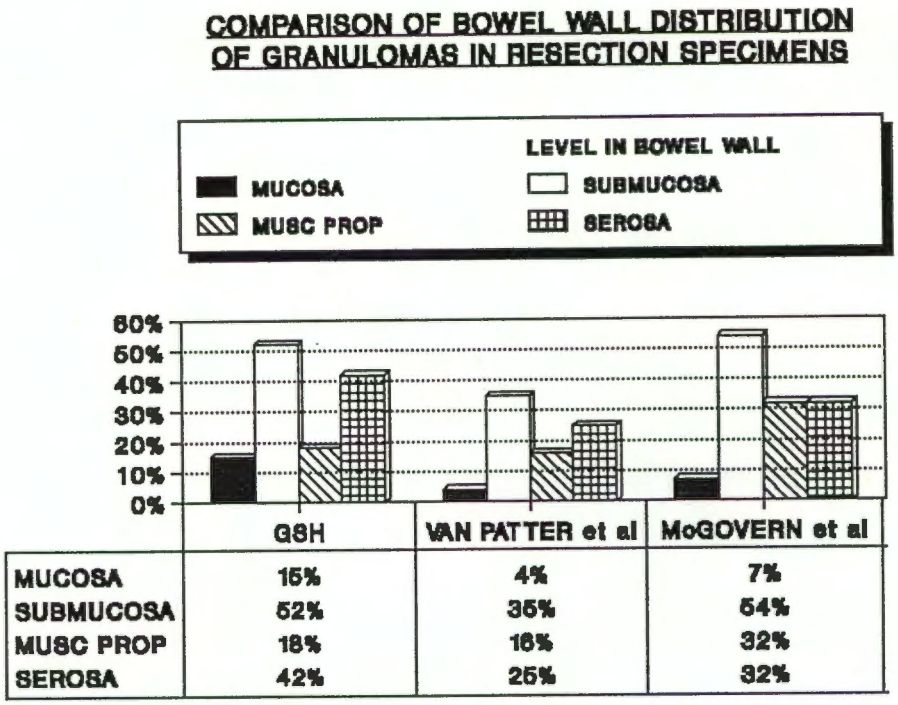
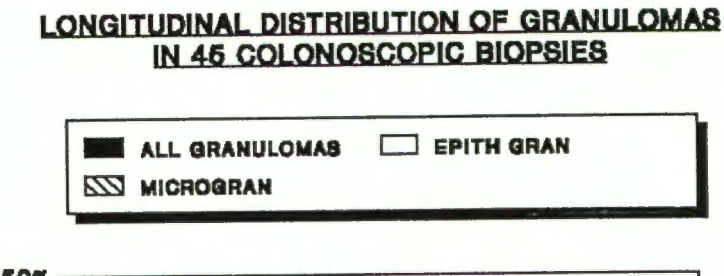


Figure 10: The pattern of distribution of granulomas in our series is very similar to Van Patter et al.(8). Their series consisted of 315 initial presenting resection specimens and 62 recurrences. The most obvious difference is in the incidence of granulomas in the mucosal layer - 15% compared with 4%. The presence of microgranulomas (which they did not evaluate) in the mucosal layer in 10% of our resection specimens would account for this.

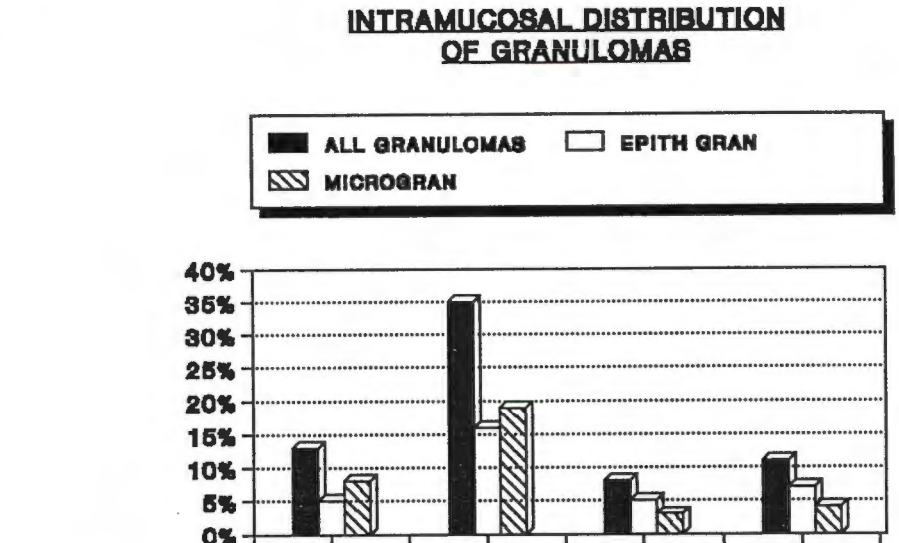
FIGURE 11:



	CAECUM	ASCEND	TRANS	DESC	SIGMOID	RECTUM
ALL GRANULOMAS	18%	35%	22%	37%	48%	35%
EPITH GRAN	9%	12%	5%	15%	25%	28%
MICROGRAN	9%	23%	17%	22%	21%	12%

Figure 11: This graph shows an apparent increase in incidence of granulomas on the left side of the colon with the highest numbers in the sigmoid and descending colon. There is an appreciable dip in the transverse colon, a finding which is supported by Schmitz-Moormann et al (40). They differed however in recording a high incidence in the caecum which we did not concur with. The low percentage on the right side is noteworthy as Crohn's disease is supposed to be mainly a right-sided disease. Other less specific inflammatory changes were found to be increased on the right as would be expected. (see Table 3).

FIGURE 12:



	CRYPT	LAM PROP	SUBMUCOSA	LYMPH FOLL
ALL GRANULOMAS	13%	35%	8%	11%
EPITH GRAN	5%	16%	5%	7%
MICROGRAN	8%	19%	3%	4%

Figure 12: By far the majority of granulomas in mucosal biopsies were situated in the lamina propria and not associated with crypts. There is also a trend for microgranulomas and epithelioid granulomas to follow each other and this would seem to indicate that they are related, in the mucosa at least, as has been suggested by others (43).

TABLE 1:

PRESENCE OF GRANULOMAS vs NCCD CLINICAL DISEASE ACTIVITY INDEX: (CDAI)

[I] EPITHELIOID GRANULOMAS:

	CDAI SCORE		
	QUIESCENT DISEASE (SCORE <=150)	ACTIVE DISEASE (SCORE >150)	
CASES WITH EPITH. GRANS	33	21	54
CASES WITHOUT EPITH. GRANS	43	51	94
TOTAL CASES	76	72	148

CHI-SQUARE = 3.86

p-VALUE < 0.15

The mean CDAI score for those patients with and without granulomas was calculated.

	MEAN CDAI SCORE (148 CASES)
EPITH. GRAN POSITIVE	142.25*
EPITH. GRAN NEGATIVE	172.48*

* - p-VALUE < 0.08

* - the difference between these 2 scores approaches statistical significance.

TABLE 1 (contd):

[II] MICROGRANULOMAS:

	CDAI SCORE		
	QUIESCENT DISEASE (SCORE <=150)	ACTIVE DISEASE (SCORE >150)	
CASES WITH MICROGRANS	19	20	39
CASES WITHOUT MICROGRANS	57	52	109
TOTAL CASES	76	72	148

CHI-SQUARE = 0.15

p-VALUE < 0.70

	MEAN CDAI SCORE (148 CASES)
MICROGRAN POSITIVE	160.97
MICROGRAN NEGATIVE	159.61

p-VALUE < 0.18

The above Tables show that there is no statistical correlation between the presence of granulomas (both epithelioid and microgranulomas) and clinical disease "activity" (CDAI). However, the mean CDAI score is much lower for patients with epithelioid granulomas than those without. This difference

approaches statistical significance and implies that those patients with epithelioid granulomas have less active disease. This does not hold true for patients with microgranulomas.

[II] COLONOSCOPY SPECIMENS:

II (i) GRANULOMAS:

FIGURE 13:

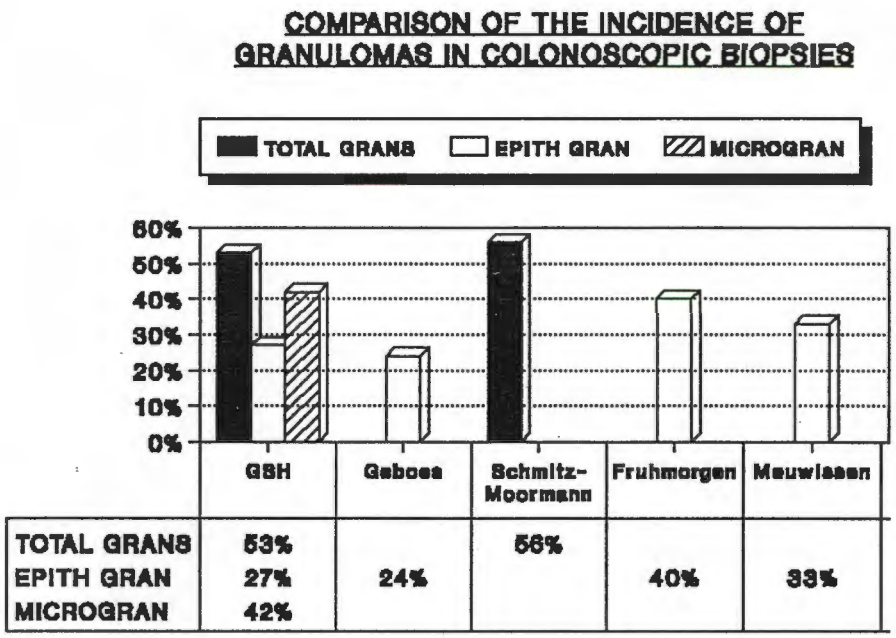


Figure 13: REFERENCES:

Geboes et al	(26)
Schmitz-Moormann et al	(40)
Fruhmorgen	(69)
Meuwissen et al	(70)

In our series, we separated the total granulomas into epithelioid granulomas and microgranulomas. Schmitz-Moormann et al are the only other series reported with microgranulomas although they did not separate them from epithelioid granulomas (40). Their total incidence was 56% - which compares favourably with our figure of 53%.

Geboes et al found epithelioid granulomas in 24% of colonoscopic biopsies (26). This differs slightly to Fruhmorgen and Meuwissen et al who obtained figures of 40% and 33% respectively (69,70).

II (ii) INFLAMMATORY CHANGES:

FIGURE 14:

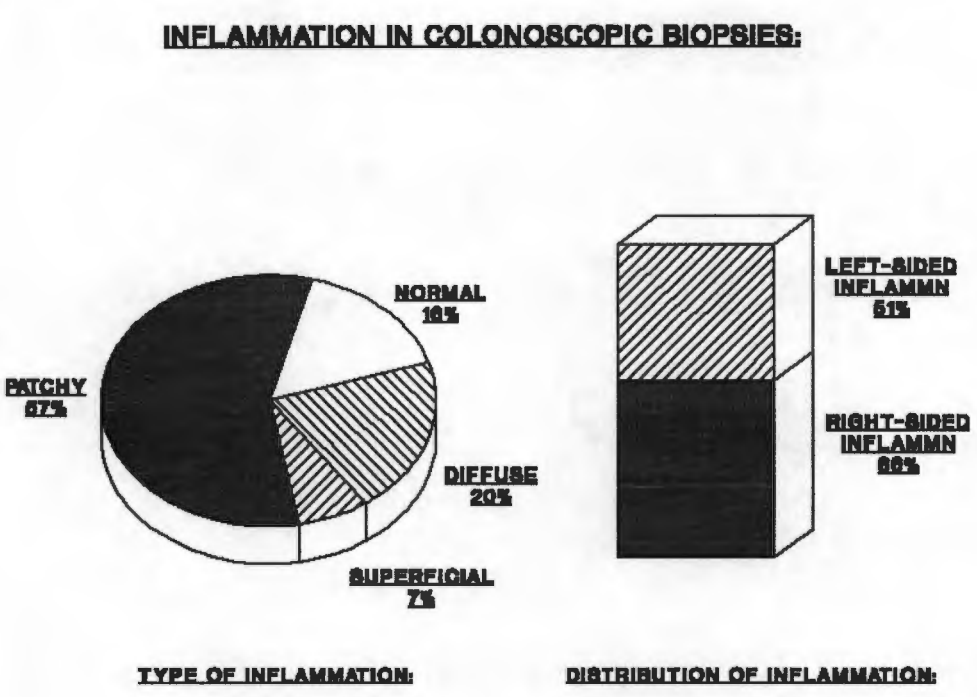


Figure 14: Only 16% of colonoscopic biopsies were considered to be normal histologically. This compares favourably with Schmitz-Moormann et al (40) who found no inflammation in 12% of their biopsies. A patchy inflammatory infiltrate, a feature which is very suggestive of Crohn's disease, was found in 57% of cases.

Inflammatory changes tend to be found more often on the right side than on the left - 66% vs 51% - although the difference between these figures is not statistically significant ($p < 0.24$). This is in keeping with the fact that Crohn's disease

is considered to affect the right side predominantly - but is in contrast to the incidence of granulomas which was found to be higher on the left side (see Figure 11).

FIGURE 15:

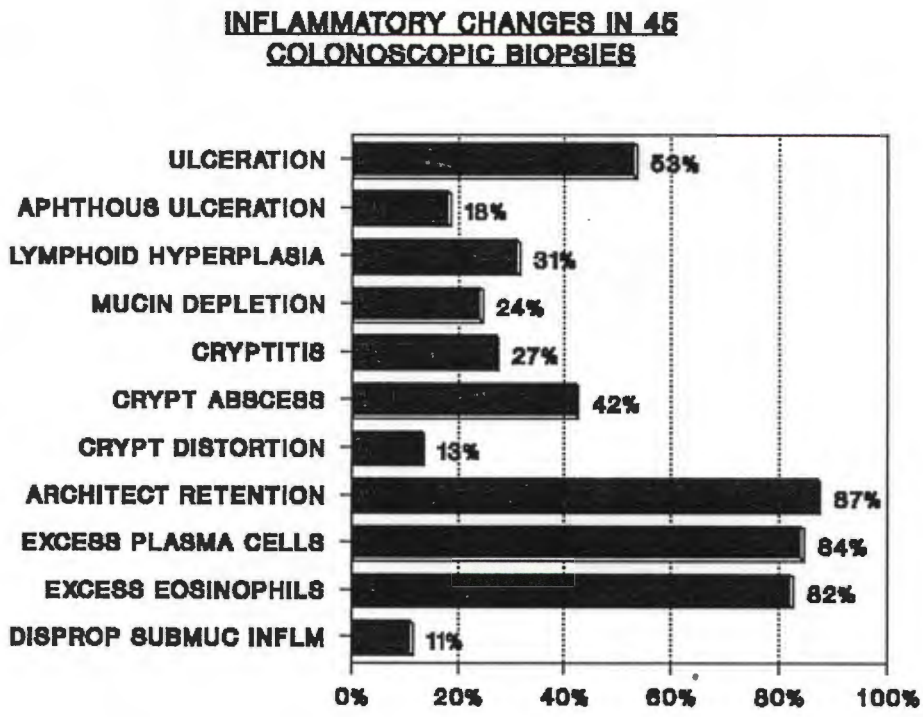


Figure 15: A large number of cases (53%) showed histologic evidence of ulceration, with aphthous ulceration, which is a feature often associated with Crohn's disease, in 18%. Of note was that crypt abscesses were found in 42% of cases, whilst distortion of crypt architecture was present in 13% - implying that 87% of cases maintained a normal architectural pattern.

TABLE 2:

II (ii) : INFLAMMATORY CHANGES IN COLONOSCOPIC BIOPSIES:

	TOTAL (45)	RIGHT COLON (32)	TRANSVERSE COLON (20)	LEFT COLON (35)
ULCERATION	53%	44%	2%	31%
APHTHOUS ULCERATION	18%	9%	0	17%
LYMPHOID HYPERPLASIA	31%	28%	2%	14%
MUCIN DEPLETION	24%	22%	2%	17%
CRYPTITIS	27%	31%	7%	6%
CRYPT ABSCESS	42%	22%	9%	34%
CRYPT DISTORTION	13%	9%	0	11%
EXCESS PLASMA CELLS	84%	88%	31%	74%
EXCESS EOSINOPHILS	82%	75%	29%	71%
DISPROPORTIONATE SUBMUC INFLAMMATION	11%	4%	0	7%

A noticeable decrease in inflammatory changes in the transverse colon is present, which tends to follow the low incidence of granulomas in this region (see Figure 11). These figures might not be representative, however, due to the small number of specimens in this group.

There is a higher incidence of aphthous ulceration in biopsies from the left colon than from the right - 17% vs 9% - although this difference is not statistically significant ($p < 0.35$). Most of the other histological parameters are more prevalent on the right side.

TABLE 3:

[II] (iii): DIAGNOSTIC VALUE OF COLONOSCOPIC BIOPSIES:

TOTAL CASES = 45

APHTHOUS ULCERATION	8	(18%)
DISPROP SUBMUCOSAL INFLAMMATION	5	(11%)
PATCHY INFLAMMATION + NORMAL ARCHITECTURE	18	(40%)
DIAGNOSTIC	24	(53%)

In the right clinical setting, other histological lesions which are characteristic of Crohn's disease include the presence of aphthous ulceration, disproportionate submucosal inflammation and a patchy inflammatory infiltrate in a biopsy with normal crypt architecture (20,28,29,30,39,60).

These features were found in 18%, 11% and 40% of cases respectively. Taking into account that some of the lesions occurred together in the same biopsy, features characteristic of Crohn's disease were found in a total of 24 cases - 53%. Thus, in just over half the biopsies, a histologic diagnosis of Crohn's disease could be confidently proposed, even in the absence of granulomas.

TABLE 4:

III (iv) : CORRELATION BETWEEN INFLAMMATORY CHANGES IN COLONOSCOPIC BIOPSIES AND THE CLINICAL DISEASE ACTIVITY INDEX (CDAI):

a) ULCERATION:

p-VALUE < 0.11

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
ULCERATION PRESENT	16	8	24
ULCERATION ABSENT	9	12	21
TOTAL CASES	25	20	45

No statistical correlation between the presence or absence of histological ulceration and clinical disease activity - ($p < 0.11$).

b) APHTHOUS ULCERATION:

p-VALUE < 0 48

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
APHTHOUS ULCERATION PRESENT	5	3	8
APHTHOUS ULCERATION ABSENT	20	17	37
TOTAL CASES	25	20	45

No statistical correlation between presence or absence of aphthous ulceration and clinical disease activity ($p < 0.48$)

c) MUCIN DEPLETION:

p-VALUE < 0.60

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
MUCIN DEPLETION PRESENT	6	5	11
MUCIN RETENTION	19	15	34
TOTAL CASES	25	20	45

No statistical correlation between mucin depletion and clinical disease activity ($p < 0.60$).

d) NEUTROPHILS IN THE LAMINA PROPRIA:

p-VALUE < 0.43

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
NEUTROPHILS PRESENT	9	5	14
NEUTROPHILS ABSENT	16	15	31
TOTAL CASES	25	20	45

No statistical correlation between the presence of neutrophils in the lamina propria (i.e. histological evidence of activity) and clinical disease activity ($p < 0.43$).

e) CRYPTITIS:

p-VALUE < 0.82

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150	ACTIVE DISEASE (SCORE >150)	
CRYPTITIS PRESENT	7	5	12
CRYPTITIS ABSENT	18	15	33
TOTAL CASES	25	20	45

No statistical correlation between cryptitis and clinical disease activity (p < 0.82).

f) CRYPT ABSCESSES:

p-VALUE < 0.79

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
CRYPT ABSCESS PRESENT	11	8	19
CRYPT ABSCESS ABSENT	14	12	26
TOTAL CASES	25	20	45

No statistical correlation between crypt abscesses and clinical disease activity (p < 0.79).

[III] RECTAL BIOPSIES:

TABLE 5:

III (i) : PRESENCE OF GRANULOMAS IN RECTAL BIOPSIES CORRELATED WITH ANATOMICAL DISTRIBUTION OF DISEASE:

DISTRIBUTION OF DISEASE INVOLVEMENT

	MACROSCOPIC APPEARANCE OF RECTUM									
	NORMAL RECTUM									RECTUM IN-VOLVED
	ILEITIS (19)	ILEO-COLITIS (19)			COLITIS (12)					
		R (9)	L (1)	T (9)	R (0)	L (1)	T (11)			
EPITH GRAN	2	1	1	4	0	0	4			4
MICROGRAN	0	0	0	0	0	1	4			2
TOTAL	2 (11%)	6 (32%)			7 (58%)					5 (45%)

R = right colonic involvement; L = left colonic involvement; T = total colon involved.

Those cases with a macroscopically normal appearing rectum were then divided according to the distribution of disease involvement into ileitis only, ileo-colitis and colitis only. The incidence of granulomas in a biopsy of normal rectum increases from 11% in patients with ileal involvement only to 58% in patients with colonic disease.

Granulomas are also more often found when there is total colonic involvement as opposed to right or left sided disease.

When the rectum is involved by the disease process, granulomas are present in 45% of cases.

The number of cases in each group is relatively small and this detracts from the significance of the results.

Microgranulomas are found mainly in left sided disease and as these are early lesions, these patients presumably present earlier in the disease course than patients with right sided disease.

FIGURE 16:

III (ii) INFLAMMATORY CHANGES IN RECTAL BIOPSIES:

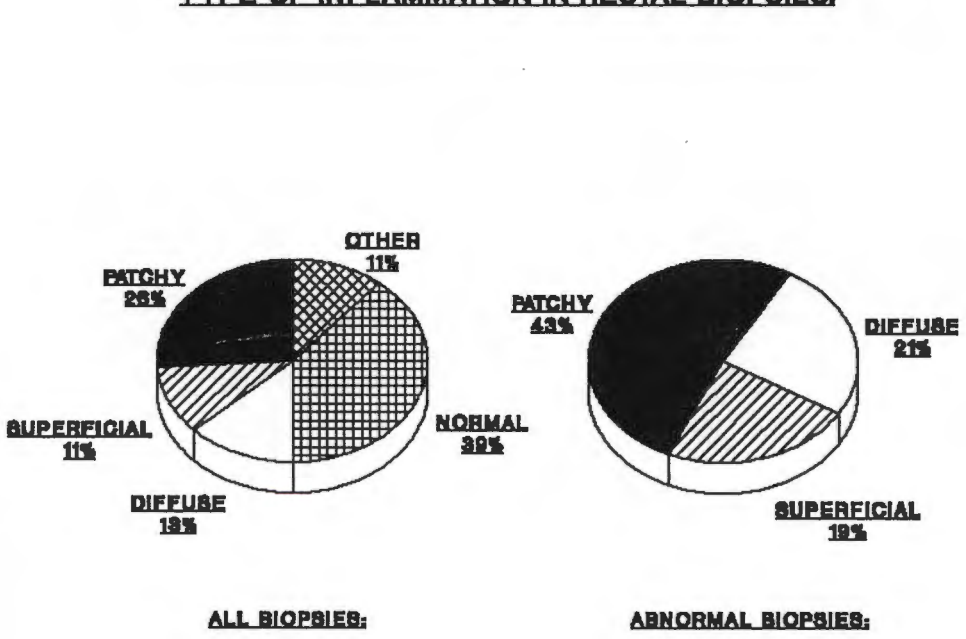


Figure 16: Of the 61 rectal biopsies 39% were considered to be normal. Inflammatory changes were present in 50%.
11% of biopsies showed no significant inflammatory infiltrate, but were not considered normal as they demonstrated other features such as aphthous ulceration, epithelioid granulomas, etc.

TABLE 6:

INFLAMMATORY CHANGES PRESENT IN 61 ISOLATED RECTAL BIOPSIES:

	NO.OF CASES	% OF ALL RECTAL BIOPSIES (61)	% OF ABNORMAL RECTAL BIOPSIES (37)
INFLAMM sup	7	11%	19%
patchy	16	26%	43%
diffuse	8	13%	21%
TOTAL	31	50%	83%
APHTHOUS ULCER	8	13%	21%
DISPROP. SUBMUC INFLAMMATION	3	5%	8%
LYMPH HYPER	8	13%	21%
CRYPTITIS	7	11%	19%
CRYPT ABSCESS	8	13%	21%
CRYPT DISTORTN	4	7%	11%
MUCIN DEPLETION	2	3%	5%

Of the 61 rectal biopsies, 50% showed inflammatory changes. A patchy inflammatory infiltrate was seen in 26%.

Aphthous ulceration was present in 13% and disproportionate submucosal inflammation in 5%.

TABLE 7:

INFLAMMATORY CHANGES IN RECTAL BIOPSIES CORRELATED WITH ANATOMICAL DISTRIBUTION OF DISEASE:

	DISTRIBUTION OF DISEASE INVOLVEMENT			
	MACROSCOPIC APPEARANCE OF RECTUM			
	NORMAL RECTUM			RECTUM IN-VOLVED
	ILEITIS (19)	ILEO-COLITIS (19)	COLITIS (12)	(11)
INFLAMM. sup	1(5%)	3(16%)	2(17%)	1(9%)
patchy	5(26%)	4(21%)	6(50%)	1(9%)
diffuse	0	2(11%)	2(17%)	4(36%)
TOTAL	6(31%)	9(47%)	10(83%)	6(55%)
APHTHOUS ULCER	0	2(11%)	4(33%)	2(18%)
DISPROP. SUBMUC INFLAMMATION	1(5%)	2(11%)	0	0
LYMPH HYPERPLASIA	2(10%)	3(16%)	1(8%)	4(36%)
CRYPTITIS	1(5%)	1(5%)	4(33%)	2(18%)
CRYPT ABSCESS	1(5%)	2(11%)	4(33%)	2(18%)
CRYPT DISTORTION	1(5%)	0	3(25%)	1(9%)
MUCIN DEPLETION	0	0	1(8%)	1(9%)

Even in macroscopically normal rectal mucosa, inflammatory changes were found in a significant number of biopsies. The incidence tended to increase from 31% in patients with disease confined to the ileum to 83% in those with colitis. Aphthous ulceration was found significantly more often in patients with colonic involvement than those with more proximal disease ($p < 0.04$).

In patients with clinical involvement of the rectum, inflammatory changes were found in 55% of cases only. The numbers of cases in each category is small, however, and this places some limitation on the value of the figures.

TABLE 8:

III (iii) : THE DIAGNOSTIC VALUE OF RECTAL BIOPSY:

NORMAL	24 (39%)	
ABNORMAL	37 (61%)	
	Non-specific	9 (15%)
	Diagnostic	28 (46%)

Diagnostic, or highly suggestive, biopsies included:-

a) the presence of granulomas
b) aphthous ulceration
c) disproportionate submucosal inflammation
d) a combination of patchy inflammation and normal crypt architecture together in the same biopsy.

Of the 61 rectal biopsies, 24 (39%) were considered to be normal. 37 (61%) showed microscopic abnormalities although only 11 (18%) were considered to be abnormal macroscopically (see Table 7).

Table 8 above, shows the number of diagnostic cases including those with granulomas.

Even in the absence of granulomas, certain features strongly favour the diagnosis of Crohn's disease.

TABLE 8A:

TOTAL NO.OF CASES = 61

APHTHOUS ULCERATION	8 (13%)
DISPROP. SUBMUCOSAL INFLAMMATION	3 (5%)
PATCHY INFLAMMATION + NORMAL ARCHITECTURE	14 (23%)
HIGHLY SUGGESTIVE	25 (41%)

Thus, even in the absence of granulomas, rectal biopsies were still highly suggestive of Crohn's disease in 41% of cases.

TABLE 8B:

DIAGNOSTIC FEATURES IN RECTAL BIOPSY vs MACROSCOPIC APPEARANCE OF THE RECTUM:

	MACROSCOPIC APPEARANCE		
	NORMAL (50)	ABNORMAL (11)	TOTAL (61)
APHTHOUS ULCERATION	6 (12%)	2 (18%)	8 (13%)
DISPROP. SUBMUCOSAL INFLAMMATION	3 (6%)	0	3 (5%)
PATCHY INFLAMMATION + NORMAL ARCHITECTURE	13 (26%)	1 (9%)	14 (23%)
HIGHLY SUGGESTIVE	22 (44%)	3 (27%)	25 (41%)

A rectal biopsy from a macroscopically normal rectum showed features highly suggestive of Crohn's disease in 44% of cases.

In an involved rectum, a biopsy was highly suggestive in 27% of cases. As only 11 biopsies of abnormal rectum were performed, there is some limitation on the value of the figures obtained.

TABLE 9:

III (iv) : CORRELATION BETWEEN INFLAMMATORY CHANGES IN RECTAL BIOPSIES AND CROHN'S DISEASE ACTIVITY INDEX (CDAI):

a) ULCERATION:

p-VALUE < 0.65	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
ULCERATION PRESENT	3	4	7
ULCERATION ABSENT	28	26	54
TOTAL CASES	31	30	61

No statistical significance between the presence of histologic ulceration and clinical disease activity (p < 0.65).

b) APHTHOUS ULCERATION:

p-VALUE < 0.48

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
APHTHOUS ULCERATION PRESENT	5	3	8
APHTHOUS ULCERATION ABSENT	26	27	53
TOTAL CASES	31	30	61

No statistical significance between aphthous ulceration and clinical disease activity (p < 0.48).

c) MUCIN DEPLETION:

p-VALUE < 0.98

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
MUCIN DEPLETION PRESENT	4	4	8
MUCIN RETENTION	27	26	53
TOTAL CASES	31	30	61

No statistical correlation between mucin depletion and clinical disease activity (p < 0.98)

d) NEUTROPHILS IN THE LAMINA PROPRIA:

p-VALUE < 0.40

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
NEUTROPHILS PRESENT	4	7	11
NEUTROPHILS ABSENT	27	23	50
TOTAL CASES	31	30	61

No statistical significance between neutrophils in the lamina propria (histologic active disease) and clinical disease activity ($p < 0.40$).

e) CRYPTITIS:

p-VALUE < 0.65

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
CRYPTITIS PRESENT	3	4	7
CRYPTITIS ABSENT	28	26	54
TOTAL CASES	31	30	61

No significant correlation between the presence of cryptitis and clinical disease activity ($p < 0.65$).

f) CRYPT ABSCESSES:

p-VALUE < 0.48

	NCCD ACTIVITY INDEX SCORE		
	QUIESCENT DISEASE (SCORE <= 150)	ACTIVE DISEASE (SCORE >150)	
CRYPT ABSCESS PRESENT	5	3	8
CRYPT ABSCESS ABSENT	26	27	53
TOTAL CASES	31	30	61

No statistical correlation between the presence of crypt abscesses and clinical disease activity ($p < 0.48$).

CHAPTER 4

DISCUSSION

[I] GRANULOMAS:

This dissertation is a retrospective study of a cohort of known Crohn's disease patients in which, well known histological indices from initial presenting pathological specimens are analysed. The single most important histological finding in the diagnosis of Crohn's disease is the epithelioid granuloma . More recently, less obvious granulomatous foci, designated microgranulomas have been described (43). This lesion is thought to possibly represent a precursor of the more mature epithelioid granuloma.

[i] Incidence of granulomas:

As the pathological specimens in this series varied from full resection specimens to endoscopic biopsies and solitary rectal biopsies, it is interesting to compare the incidence of granulomas in the different material available (Figure 7).

Most figures quoted in the literature are obtained from analysing resection specimens and vary from 36 per cent of cases to 75 per cent (Figure 8). Most of the specimens examined included both small and large intestine. None of

these series, however, took into account the presence of microgranulomas - seen in 27 per cent of our resection specimens. By including this feature, the incidence of granulomas in resection specimens increased from 68 per cent to 76 per cent.

(ii) Distribution of granulomas in the layers of the intestinal wall:

The distribution of granulomas within the bowel wall shows that the highest incidence occurs in the submucosa and that the mucosa has the lowest incidence of epithelioid granulomas.(Figure 9). A high incidence of granulomas is also found in the serosal layer. However there is a noticeable decrease in incidence in the muscularis propria. This could be due to a reduction in lymphatic drainage and lymphoid follicles in the muscular layer and, in our experience and that of other authors, granulomas in Crohn's disease are often associated with lymphatic vessels (8,65). The lymphatic drainage of the muscularis propria has been shown to be derived from the submucosal plexus. Lymph vessels run through the muscularis externa where they receive tributaries from the lymphatic plexus in the muscular coat and then follow the blood vessels into the retroperitoneal tissue (71). Van Patter et al found lymphoid follicles in the muscularis propria in only 15.7% of their cases, whilst McGovern and Goulston found them in 71% of cases (8,53). Van Patter et al also felt that

granulomas in the muscular layer appeared to develop and regress later than those in other layers (8). The large discrepancy in the incidence of lymphoid follicles and the decrease in granulomas in the muscularis propria are not easy to explain fully, but are posibly related to the length of time of the disease process.

Microgranulomas were only found in the mucosa and not in any other layers of the bowel wall (Figure 9). This finding has not previously been well documented in the literature. The microgranuloma is thought to be an early precursor lesion of the more mature epithelioid granuloma (43), but as they are not found along with mature granulomas in the other layers of the intestine, it is possible that in the deeper layers, the two are not related.

Isolated giant cells occurred throughout the bowel wall and were often present in conjunction with fissuring ulceration. A reasonable assumption then would be that they represent a foreign body type of response to ingested material and are a non-specific finding.(1,39,62)

(iii) Longitudinal distribution of granulomas in the large intestine:

The longitudinal distribution of granulomas in the large intestine is another interesting feature. Chambers and Morson found a low incidence in the ileum with increasing frequency in colon, rectum and anus (50). They postulated that this may be due to environmental differences or differences in tissue susceptibility. Schmitz-Moormann et al, in a study of colonic and rectal biopsies found the highest frequency of granulomas in the caecum and descending colon and the lowest values were seen in the transverse colon and rectum (40).

Our figures for colonoscopic biopsies show that within the colon granulomas were most often found on the left side of the colon - in the sigmoid and descending colon - and least often found in caecum and transverse colon (Figure 11). This is at slight variance with the general perception that Crohn's disease affects mainly the right side of the colon. Other less specific inflammatory features were, however, found more often on the right side than the left (Figure 14). The identification of regressed granulomas is difficult (8), but if these were shown to be present in the right side of the colon, it could be suggested that the regional variation is related to the length of time until presentation. As with caecal carcinoma, which classically remains occult longer than more distal tumours, right sided Crohn's disease may manifest or require surgical intervention at a later stage in the

disease course than left sided involvement and that by this time granulomas are less obvious histologically (40,50,51).

(iv) Intramucosal distribution of granulomas:

In mucosal biopsies, the highest incidence of granulomas occurred in the lamina propria (Figure 13). Some authors have suggested that granulomas in Crohn's disease are a secondary phenomenon and not part of the primary pathological process (8), whilst others have alluded to the fact that the initial event in the formation of a mucosal granuloma seems to be the aggregation of lymphocytes at the base of a crypt. The crypt epithelium then disintegrates and ruptures with formation of a granuloma around degenerating epithelium (46,53). Our findings were that in the mucosa only 13 per cent of granulomas were associated with crypts and 35 per cent were isolated in the lamina propria, although serial sectioning of the biopsies was not performed routinely. This would infer that the formation of granulomas does not correspond to disintegration of crypt epithelium, but is aligned to some other aetiological process.

A number of authors have documented the relationship of granulomas to lymphoid tissue and lymphatics (8,51,65). This may be true in the deeper layers of the intestinal wall, but it is a well known feature that the mucosal layer does not contain lymphatics (72). Also, in our series we did not find significant numbers of granulomas associated with lymphoid

follicles in the mucosa (Figure 12). Another interesting feature seen in Figure 12 is the trend for microgranulomas to follow the incidence of epithelioid granulomas. This trend does not appear in the other layers of the intestinal wall (see Figure 9).

The interpretation of these findings seems to suggest that granulomas in the mucosal layer are somehow different to those found in other layers of the intestinal wall - and that in this layer, microgranulomas may be found as precursor lesions.

(v) Correlation of the presence of granulomas with the Crohn's Disease Activity Index (CDAI):

The question of the relationship of granulomas to clinical course is a vexing one. In this study, granulomas were compared to the NCCD clinical index (58). Previous series have used different indices when assessing the relationship of granulomas to clinical prognosis. Van Patter et al (8), Antonius et al (52), Gump et al (54) and Wolfson et al (55) compared the presence of granulomas in resection specimens to the recurrence rate. They found no correlation between the two. Ward and Webb (42) assessed the usefulness of rectal biopsies as an indicator of prognosis in Crohn's proctocolitis. Their material included patients with disease limited to the colon who had rectal biopsy material available for review. The clinical outcome of the patients was assessed as being good (asymptomatic), moderate (symptomatic),

requiring colectomy and death from disease. They found no correlation between the presence of granulomas and clinical outcome. However, they found that there was an increase in degree of histological abnormality in biopsies of patients who subsequently died of granulomatous colitis. They also showed that the presence of ulceration and fissures histologically correlated with a bad prognosis.

In 1976, Glass and Baker reported in their series on the role of the granuloma in recurrent Crohn's disease that the presence of granulomas within a specimen at primary excision appeared to offer a better prognosis (56). Again, they compared resection specimens against evidence of recurrence displayed by either further operation and histological or radiological evidence of disease. Symptomatic evidence of recurrence alone was not accepted. These findings conflicted with the previous reports mentioned. Chambers and Morson followed this with a paper representing an attempt to relate a quantification of granulomas in bowel resected for Crohn's disease with the subsequent course of the disease (50). They assessed the subsequent course of the disease by dividing the patients into four groups : those with no evidence of recurrent disease, those with radiological or biopsy evidence in previously uninvolved bowel, those who required surgery and those whose symptoms and signs persisted postoperatively for more than six months. By quantitating the number of granulomas per section examined, they found that the group with no

recurrence had significantly more granulomas per section than those patients whose disease persisted or recurred after surgery. This was in general agreement with the findings of Glass and Baker (56).

A slightly different approach was pursued in this study, in that an attempt was made to compare histological lesions with the Crohn's Disease Activity Index (CDAI) (58). By using this index an assessment of disease activity at the time of biopsy was obtained and thus one would expect this to correlate with the microscopic findings, irrespective of whether the patient was on treatment or not. The statistical analysis (chi-square and p-values) showed no correlation between the presence or absence of granulomas and the NCCD index (Table 4). However, what was of interest was that the mean NCCD score for patients with granulomas was 142.25 and the mean score for patients without granulomas was 172.48. The difference between these figures approaches statistical significance ($p < 0.08$) and this would suggest that patients with granulomas have less disease activity and therefore are less likely to relapse. If correct, these findings would tend to support the conclusions of Chambers and Morson and Glass and Baker (50,56)

[II] COLONOSCOPY SPECIMENS:

(i) Granulomas:

Colonoscopy has provided increasing amounts of material for histological evaluation in patients with Crohn's disease. Recognising the limitations in interpreting mucosal biopsies, the presence of granulomas is still of major importance (39). 53 per cent of the colonoscopic biopsies in this series contained granulomas. Although this is noticeably higher than the incidence quoted in some series in the literature (figure 13), the latter figures were limited to the presence of epithelioid granulomas only and did not take microgranulomas into account. Schmitz-Moormann et al, in a similar series found both epithelioid and microgranulomas in 56% of cases (40).

Figure 13 shows that 27 per cent of cases contained epithelioid granulomas (which compares favourably with other series) and in 42 per cent microgranulomas were present (26,69,70). We did not differentiate as to whether the biopsies obtained were from macroscopically normal or abnormal bowel (26,39).

These figures show that the presence of microgranulomas is of considerable importance and adds significantly to the diagnostic criteria for Crohn's disease.

(ii) Inflammatory changes:

Figure 14 indicates that 84 per cent of all colonoscopic biopsies in our series were considered to be abnormal and there was a higher incidence of abnormalities of the right side of the colon compared to the left - 66 per cent versus 51 per cent. Although this difference is not statistically significant ($p < 0.24$), this is still a well recognised feature of Crohn's disease (5). In a similar series, 12 per cent of colonic biopsies showed no evidence of inflammation (40). The high incidence of abnormalities in colonoscopic biopsies could be biased by the endoscopists tendency to biopsy macroscopically abnormal areas. However, one of the distinguishing features of Crohn's disease is the focality of the process and one would expect this to be reflected in the histology as well. At the level of the individual biopsy, a patchy inflammatory infiltrate was found in 57 per cent of colonic biopsies with a diffuse infiltrate in 20 per cent (Figure 14).

Figure 15 shows the incidence of other inflammatory changes present. Mucin depletion, or loss of goblet cells and crypt architectural distortion - characteristics which are said to be more typical of ulcerative colitis than Crohn's disease - were found in 24 per cent and 13 per cent of colonoscopic biopsies respectively. Distortion of the crypt architecture will, however, be present at the edge of ulcers and so endoscopy bias is important in interpreting this result.

Of note was the presence in 42 per cent of cases of crypt abscesses which have been said to be commoner with ulcerative colitis than Crohn's disease. They are of more use as an index of histological activity than in differentiating between these two inflammatory bowel diseases.

On examining the inflammatory changes according to their anatomical location in the colon, one sees that most of the changes are more prevalent on the right side (Table 2).

One of the noticeable exceptions to this is the presence of aphthous ulceration, which is considered to be one of the earliest identifiable histological lesions of Crohn's disease (18,20). This erosive or ulcerating lesion is often related to a mucosal or submucosal lymphoid aggregate and the first stage in the development of an ulcer appears to be degeneration of the basal cells of the mucosal tubules immediately overlying a lymphocytic accumulation which is the area of antigen exchange (53). An acute inflammatory exudate develops and extension of this process presumably results in the development of fissures and fistulae. Early aphthous ulceration was found in 18 per cent of colonoscopic biopsies (Figure 15, Table 2) - 17 per cent occured on the left side and 9 per cent on the right side. These findings would correlate with the assumption that the disease process is initiated in the terminal ileum with early progression to the right side of the colon (50,65). Left sided involvement occurs later in the disease process, often

by the time the patient presents clinically and therefore early changes are more likely to be present in biopsy specimens of this region. This proposition is compatible with the explanation given for the longitudinal distribution of granulomas, as discussed earlier.
However, a more simple explanation might be that the results are related to the accessibility of the regions of the colon to the endoscopist and it is easier to visualise and biopsy the left side than the right.

Another feature of the anatomical distribution of inflammatory changes in our series is the generally low incidence of abnormalities in the transverse colon. This holds true for both granulomas and other inflammatory lesions (Figure 11 and Table 2). There appears to be an unexplained sparing effect of the transverse colon by the disease process. Schmitz-Moormann et al found a similar low incidence of granulomas in the transverse colon, but did not find other inflammatory changes to be significantly reduced in this region (40).

(iii) Diagnostic value of colonoscopic biopsies:

The development of the flexibile fibreoptic colonoscope has increased the amount of material available for the histopathologist to examine. Only limited patterns of injury, however, can be revealed in biopsy samples (28,39). As granulomas are only found in about half of colonoscopic

biopsies, other less specific inflammatory changes need to be closely scrutinized in the diagnosis of Crohn's disease. Pathologists in general are very wary about confidently diagnosing Crohn's disease in the absence of granulomas and are often tempted to interpret a biopsy as only showing "non-specific inflammation".

Although many features have been described in biopsies of Crohn's disease, most in isolation do not help in distinguishing it from other forms of colitis (20,29,60). Apart from the presence of granulomas, findings, which in the right clinical setting, strongly favour the diagnosis of Crohn's disease include : aphthous ulceration (18,20), disproportionate submucosal inflammation (20,28,29,30,36,45,53), a focal or patchy inflammatory infiltrate with maintenance of the normal crypt architecture (30,39,60) and a normal rectal biopsy in a definite case of "colitis" (38).

Table 3 demonstrates that in 24 cases (53%) one or more of these features were present. Thus, one would be reasonably confident in making a histological - or at least supporting a clinico-radiological - diagnosis of Crohn's disease in just over half the cases, even in the absence of granulomas.

(iv) Correlation of inflammatory changes with the Crohn's Disease Activity Index (CDAI):

In order to evaluate therapeutic trials in Crohn's disease it has been important to assess disease activity in patients. By using the NCCD clinical index, we attempted to correlate the presence or absence of certain histological findings in colonoscopic biopsies with the physician's assessment of activity (Table 4). None of the histological features of active colitis showed significant correlation with clinical evidence of active disease. This significant negative finding would imply that the Crohn's Disease Activity Index is a relatively inaccurate method of assessing disease severity at the tissue level.

A previous study relating disease activity indices with colonoscopic findings obtained similar results (62).

[III] RECTAL BIOPSIES:

(i) Granulomas:

Rectal biopsy is one of the simplest and most frequently performed investigations in the diagnosis of Crohn's disease. In the rectal biopsies of this series, granulomas were found in a total of 33 per cent of cases, with epithelioid granulomas in 26 per cent and microgranulomas in 12 per cent (Figure 7). The significance and value of these figures,

though becomes more apparent when one analyses the incidence of granulomas in conjunction with the extent and distribution of bowel involved by the disease process (Table 5). The closer the disease process approaches the rectum, the more likely one is to find granulomas in a rectal biopsy (28). In those patients with disease confined to the ileum alone, biopsy of the normal appearing rectum revealed granulomas in 11 per cent of cases. 32 per cent of rectal biopsies from patients with ileo-colitis and a macroscopically normal rectum and 58 per cent of cases with colitis and a normal rectum, contained granulomas.

Biopsy of a macroscopically involved rectum, revealed granulomas in 45% of cases

(ii) Inflammatory changes:

If one looks at all the rectal biopsies, 24 out of 61 (39 per cent) were considered to be normal microscopically and 37 (61 per cent) showed some abnormality (Figure 16).

An inflammatory infiltrate was present in 50 percent of biopsies with a patchy infiltrate present in 26 per cent.

Mucin depletion and crypt architectural distortion, which are more often associated with ulcerative colitis, were only found in a minority of cases whilst crypt abscesses were much less common than in the colon (Table 6).

As with granulomas, inflammatory changes in rectal biopsies increase in incidence as the disease process approaches the rectum (Table 7). The fact that only 55% of cases with macroscopic disease of the rectum showed microscopic inflammatory features, confirms again that macroscopic findings do not correlate well with changes seen on tissue level (62).

Inflammatory changes are present in a number of rectal biopsies even when the disease process is limited to other areas of the bowel (Table 7). Non-specific inflammatory changes were found in 50 per cent of all rectal biopsies. Almost one-third of rectal biopsies in patients with disease limited to the ileum, showed inflammatory changes. 47 per cent of cases with ileo-colitis had inflammatory changes in a rectal biopsy, whilst this figure increased to 83 per cent when the patient had predominantly left-sided colitis and a normal rectum (Table 7). Thus the presence of microscopic rectal inflammatory changes is generally related to the proximity of grossly demonstrable disease. This finding has been documented before (28,44).
Similarly, a statistically significant increase in aphthous ulceration was found in biopsies from macroscopically normal rectums in patients with colonic involvement, compared to patients with more proximal disease ($p < 0.04$) (Table 7).

(iii) Diagnostic value of rectal biopsy:

Rectal biopsy is a relatively simple technique with few complications (47). Yet, its value in the diagnosis of Crohn's disease is still controversial. In the presence of rectal disease, Lockhart-Mummery and Morson were able to confirm the diagnosis in 16 out of 19 cases (61). However, Korelitz and Sommers found histologic attributes of Crohn's disease in 30 per cent of rectal biopsy specimens of a sigmoidoscopically normal appearing rectal segment (44). Dyer et al found diagnostic features in 23 per cent of rectal biopsies (28). Some authors recommend serial sectioning of rectal biopsies to increase the diagnostic yield (35,36). However, this is a time consuming procedure and is not always easy to perform in busy surgical diagnostic laboratories.

In contrast to these reports, Anderson and Bogoch found non-specific inflammatory changes in 43 per cent of rectal biopsies in patients with Crohn's ileitis, but no granulomas were seen (41). In their series of patients with Crohn's colitis, McGovern and Goulston found rectal biopsy unhelpful (53). Hill et al examined a large amount of material from the National Cooperative Crohn's Disease Study group and found that a single random rectal biopsy was of extremely limited diagnostic use in Crohn's disease (27).

As can be seen, divergent views abound as to whether rectal biopsy is of benefit or not. Our figures show that, taking 61

rectal biopsies, diagnostic or highly suggestive features were seen in 28 of the cases (46 per cent) (Table 8) (this figure includes the presence of granulomas).

In the absence of granulomas, inflammatory changes highly suggestive of Crohn's disease were present in 25 cases (41%) in total (Table 8A). When the rectum was involved macroscopically (11 cases) these features were found in 27 per cent (Table 8B). An intriguing stastistic is that even in a macroscopically normal appearing rectum, helpful features in confirming the diagnosis were found in 44 per cent of cases (Table 8B).

The question remains as to the usefulness of rectal biopsy in the diagnosis of Crohn's disease and whether it should be performed routinely even in a normal appearing rectum. Our figures would seem to indicate that a routine biopsy of the rectum still appears to be a worthwhile procedure in the diagnosis of Crohn's disease and tends to disagree with Hill et al's conclusions (27). Even a normal rectal biopsy may still be of value, as this finding in the presence of a definite case of colitis is very suggestive of Crohn's disease (38).

(iv) Correlation of inflammatory changes with the CDAI:

Once again comparison of the microscopic findings in rectal biopsies, including the presence of ulceration, aphthous ulceration, mucin depletion, "active" inflammation such as

cryptitis and crypt abscesses with the NCCD activity index revealed no statistical correlation. (Table 9A - F). These findings are similar to those found in colonoscopic biopsies.

CONCLUSIONS:

This dissertation has set out to document a number of histological findings in the initial presenting pathological specimen in a group of known Crohn's disease patients. A mixture of resection specimens, colonoscopic and isolated rectal biopsies have been carefully studied. A control group of an equal number of specimens from unselected patients without Crohn's disease has not been a part of this study and this clearly does not allow us to make comparisons with other types of inflammatory bowel disease; however, conclusions that do not depend on such comparisons are valid and are the subject of this dissertation.

Important new findings recorded here include the fact that microgranulomas are confined to the mucosal layer of the intestine and were not demonstrated in any other layers. Although previous authors have concluded that they are precursors to the more mature epithelioid granulomas, our results suggest that at least in the deeper layers they seem to be unrelated (43). This might be explained by the fact that there are two types of granulomas in Crohn's disease and the formation of granulomas in the mucosal layer is somehow

different to that in other layers of the intestinal wall. The aetiology and pathogenesis of both granulomas and microgranulomas needs further research and elucidation.

The clinical course of Crohn's disease tends to be a progressive one and microscopic features which might predict clinical behaviour are of obvious importance. In our series we found no statistical correlation between any of the microscopic features examined and the Crohn's Disease Activity Index. The usual histological features that pathologists recognise as indicating microscopic evidence of disease activity therefore correlate very poorly with clinical assessment of disease activity and new criteria may need to be designed to indicate "active" disease.

The value of rectal biopsy in the diagnosis of Crohn's disease has been extensively investigated (27-30,36,41-47,63,66,67). Our findings endorse the view of those who feel that this is a useful procedure and we would encourage physicians to perform rectal biopsy even in the situation where the rectum appears normal macroscopically.

Despite all the results described in this dissertation, it still remains a basic principle that accurate diagnoses in medicine can only be made with good cooperation between all the disciplines involved. This truly applies to the enigma of Crohn's disease.

CHAPTER 5

REFERENCES

1. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. *J Am Med Assoc* 1932;99:1323-1329.
2. Carson HW. The iliac passion. *Annals of Medical History* 1941;3:638-694.
3. Banerjee AK, Peters TJ. The history of Crohn's disease. *J of the Royal College of Physicians of London* April 1989;23:121-124.
4. Dalziel TK. Chronic interstitial enteritis. *Br Med J* 1913;2:1068-1070.
5. Lockhart-Mummery HE, Morson BC. Crohn's disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. *Gut* 1960;1:87-105
6. Jones-Williams W. Histology of Crohn's syndrome. *Gut* 1964;5:510-516.
7. Lind E, Fansa K, Elgjo K, Gjone E. Crohn's disease - diagnostic procedures and problems. *Scand J Gastroenterol* 1985;20:660-664.
8. Van Patter WN, Bargen JA, Dockerty MB, Feldman WH, Mayo CW, Waugh JM. Regional enteritis. *Gastroenterology* 1954;26 No3:347-450.
9. Ward M. The pathogenesis of Crohn's disease. *Lancet* 1977;2:903-905.

10. Tysk C, Lindberg E, Jarnerot G, Floderns-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988;29:990-996.

11. Biemond I, Burnham WR, D'Amaro J, Langman MJS. HLA-A and -B antigens in inflammatory bowel disease. *Gut* 1986;27:934-941.

12. Hampson SJ, McFadden JJ, Herman-Taylor J. Mycobacteria and Crohn's disease. *Gut* 1988;29:1017-1019.

13. Somerville KW, Logan RFA, Edmond M, Langman MJS. Smoking and Crohn's disease. *Br Med J* 1984;289:954-956.

14. Aronson PA, Cook SL, Roche JK. Sensitisation of epithelial antigens in chronic mucosal inflammatory bowel disease. *J Immunol* 1983;131:2796-2804.

15. Hollander D, Vadheim CM, Brettholz E, Peterson GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. *Ann Intern Med* 1986;105:883-885.

16. Jewell DP. Aetiolgy and pathogenesis of ulcerative colitis and Crohn's disease. *Postgrad Med J* 1989;65:718-719.

17. Wakefield AJ, Sawyer AM, Dhillon AP, Pittilo RM, Rowles PM, Lewis AAM, Pounder RE. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* Nov4 1989;1:1057-1062.

18. Gilmour HM: In Gastrointestinal and Oesophageal Pathology. Edited by R. Whitehead. Churchill Livingstone. Edinburgh London Melbourne New York. 1989

19. Dourmashkin RR, Davies H, Wells C, Shah D, Price A, O'Morain C, Levi J. Epithelial patchy necrosis in Crohn's disease. *Hum Pathol* 1983;14:643-648.

20. Morson BC. Histopathology of Crohn's disease. *Scand J Gastroenterol* 1971;6:573-575.

21. Brooke BN. Granulomatous disease of the intestine. *Lancet* 1959;Nov7 2:745-749.

22. Vantrappen G, Agg HO, Ponette E, Geboes K, Bertrand PH. Yersinia enteritis and enterocolitis: gastroenterological aspects. *Gastroenterology* 1977;72:220-227.

23. Talbot IC, Price AB. In: Biopsy pathology in colorectal disease. London. Chapman and Hall. 1987.

24. Dvorak AM, Connell AB, Dickerseh GR. Crohn's disease: a scanning electron microscopic study. *Hum Pathol* 1979;10:165-177.

25. Dvorak AM, Silen W. Differentiation between Crohn's disease and other inflammatory conditions by electron microscopy. *Ann Surg* 1985;201:53-63.

26. Geboes K, Vantrappen G. The value of colonoscopy in the diagnosis of Crohn's disease. *Gastrointestinal Endoscopy* 1975;22:18-23.

27. Hill RB, Kent TH, Hansen RN. Clinical usefulness of rectal biopsy in Crohn's disease. *Gastroenterology* 1979;77:938-944.

28. Dyer NH, Stansfield AG, Dawson AM. The value of rectal biopsy in the diagnosis of Crohn's disease. *Scand J Gastroenterol* 1970;5:491-496.

29. Korelitz BI, Sommers BC. Differential diagnosis of ulcerative and granulomatous colitis by sigmoidoscopy, rectal biopsy and cell counts of rectal mucosa. *Am J Gastroenterol* 1974;61:460-469.

30. Morson BC. Rectal biopsy in inflammatory bowel disease. *N Eng J Med* 1972;287:1337-1339.

31. Sommers SC, Korelitz BI. Mucosal cell counts in ulcerative and granulomatous colitis. *Am J Clin Pathol* 1975;63:359-365.

32. Heatley RJ, James PD. Eosinophils in rectal mucosa. *Gut* 1978;20:787-791.

33. Symonds DA. Paneth cell metaplasia in diseases of the colon and rectum. *Arch Pathol* 1974;97:343-347.

34. Wolfson DM, Sachar DB, Cohen A. Do granulomas affect postoperative recurrence rates in Crohn's disease? (abstr). *Gastroenterology* 1981;80:1319

35. Schmitz-Moormann P, Pittner PM, Sangmeister M. Probability of detecting a granuloma in a colorectal biopsy of Crohn's disease. *Path Res Pract* 1984;178:227-229.

36. Surawicz CM, Meisel JL, Ylvisaker T, Sanders DR, Rubin CE. Rectal biopsy in the diagnosis of Crohn's disease: value of multiple biopsies and serial sectioning. *Gastroenterology* 1981;81:66-71.

37. Morson BC, Dawson IMP. Gastrointestinal Pathology. Blackwell Scientific Publications. Oxford London Edinburgh Melbourne. 3rd Edition. 1990.

38. Price AB, Morson BC. Inflammatory bowel disease: the surgical pathology of Crohn's disease and ulcerative colitis. *Hum Pathol* 1975;6:7-29.

39. Goldman H, Antonioli DA. Mucosal biopsy of the rectum, colon and distal ileum. *Hum Pathol* 1982;13:981-1012.

40. Schmitz-Moormann P, Pittner PM, Malchow H, Brandes JW. The granuloma in Crohn's disease: a bioptical study. *Path Res Pract* 1984;178:467-476.

41. Anderson FH, Bogoch A. Biopsies of large bowel in regional enteritis. *Can Med Ass J* 1968;98:150-153.

42. Ward M, Webb JN. Rectal biopsy as a prognostic guide in Crohn's colitis. *J Clin Pathol* 1977;30:126-131.

43. Rotterdam H, Korelitz BI, Sommers SC. Microgranulomas in grossly normal rectal mucosa in Crohn's disease. *Am J Clin Pathol* 1977;67:550-554.

44. Korelitz BI, Sommers SC. Rectal biopsy in patients with Crohn's disease. *JAMA* 1977;237:2742-2744.

45. Gear EV, Dobbins WO. Rectal biopsy: a review of its diagnostic usefulness. *Gastroenterology* 1968;55:522-544.

46. Goulston SJM, McGovern VJ. The value of rectal biopsies. *Med J Aust* 1972;1:1234-1238.

47. Morson BC. In: Pathology Annual. Edited by SC Sommers. Englewood Cliffs., NJ Prentice-Hall, Inc 1974 pp209-230.

48. Goodman MJ, Skinner JM, Truelove S. Abnormalities in the apparently normal bowel mucosa in Crohn's disease. *Lancet* 1976;2:275-278.

49. Petri M, Poulsen SS, Christensen K, Jarnum S. The incidence of granulomas in serial sections of rectal biopsies in patients with Crohn's disease. *Acta Pathol Microbiol Immunol Scand Sect a* 1982;90:145-147.

50. Chambers TJ, Morson BC. The granuloma in recurrent Crohn's disease. *Gut* 1976;17:75-77.

51. Hadfield G. The primary histological lesion of regional ileitis. *Lancet* 1939;2:773-775.

52. Antonius JI, Gump FE, Lattes R, Lepore M. A study of certain microscopic features in regional enteritis and their possible prognostic significance. *Gastroenterol* 1960;38:889-905.

53. McGovern VJ, Goulston SJM. Crohn's disease of the colon *Gut* 1968;9:164-176.

54. Gump FE, Sakellariadis P, Wolff M, Broell JR. Clinical-pathological investigation of regional enteritis as a guide to prognosis. *Ann Surg* 1972;176:233-242.

55. Wolfson DM, Sachar DB, Cohen A, Goldberg J, Styczynski R, Greenstein AJ, Gelernt IM, Janowitz HD. Granulomas do not affect postoperative recurrence rates in Crohn's disease. *Gastroenterology* 1982;83:405-409.

56. Glass RE, Baker WNW. Role of the granuloma in recurrent Crohn's disease. *Gut* 1976;17:75-77.

57. O'Keefe EA, Wright JP, Froggat J, Cuming A. A medium term follow up of Crohn's disease in Cape Town. *S Afr Med J* 1989;76:139-141.

58. Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. *Gastroenterology* 1976;70:439-444.

59. Binder V, Hendricksen C, Kreiner S. Prognosis in Crohn's disease - based on results from a regional patient group from the county of Copenhagen. *Gut* 1985;26:146-150.

60. Cook MG, Dixon MF. An analysis of the reliability of detection and diagnostic value of various pathological features in Crohn's disease and ulcerative colitis. *Gut* 1973;14:255-262.

61. Lockhart-Mummery HE, Morson BC. Crohn's disease of the large intestine. *Gut* 1964;5:493-509.

62. Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with chronic inflammatory bowel disease. *Gut* 1986;27:92-95.

63. Present DH, Chapman MI, Cohen N, Janowitz HD. The correlation of sigmoidoscopy and rectal valve biopsy in granulomatous disease of the small bowel (abstr). *Gastroenterology* 1967;52:1113.

64. Cornes JS, Stecher M. Primary Crohn's disease of the colon and rectum. *Gut* 1961;2:189-201.

65. Warren S, Sommers SC. Cicatrizing enteritis (regional enteritis) as a pathologic entity. *Am J Pathol* 1948;24:475-501.

66. Iliffe GD, Owen DA. Rectal biopsy in Crohn's disease. *Dig Dis Sci* 1981;26:321-324.

67. Goodman MJ, Kirsner JB, Riddell RR. Usefulness of rectal biopsy in inflammatory bowel disease. *Gastroenterology* 1977;72:952-956.

68. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. Statistical Package for the Social Sciences. McGraw-Hill, 2nd Edition. 1975.

69. Fruhmorgen P. Diagnosis of inflammatory diseases of the colon by coloscopy. *Acta Gastro-Enterol Belg* 1974;37:154-158.

70. Meuwissen SGM, Pape KSSB, Agenant D, Oushoorn HH, Tytgat GNJ. Crohn's disease of the colon. Analysis of the diagnostic value of radiology, endoscopy and histology. *Am J of Dig Dis* 1976;21:81-88.

71. Bloom W, Fawcett DW. In: A Textbook of Histology. W.B.Saunders 10th Edition 1975.

72. Fenoglio CM, Kaye GI, Lane N. Distribution of human colonic lymphatics in normal, hyperplastic and adenomatous tissue. *Gastroenterology* 1973;64:51-56.